ADAPTABILITY POTENTIAL OF *JUSTICIA ADHATODA* L. TO XERIC ENVIRONMENTS THROUGH ROOT MORPHO-PHYSIOLOGICAL AND ARCHITECTURAL MODULATIONS

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Abstract. *Justicia adhatoda* colonizes tropical and subtropical areas of the world. Populations of *Justicia adhatoda* were collected from different environments in Punjab, Pakistan. The habitats were classified into least dry habitats**,** moderately dry habitats, and severely dry habitats based on Budekyo lettu dryness ratio. These ecotypes of *Justicia adhatoda* were evaluated for adaptability potential under drought stress in relation to soil physico-chemical and environmental attributes. Root fresh weight, root dry weight and root length was significantly increased at severely dry habitats. Among root ionic contents Na^+ content was the maximum at severely dry habitats. Root Ca, K, P and Mg contents were high at Barakhu (BAR) and Khewra (KHW) sites. Among anatomical attributes, root epidermal thicknesses, epidermal cell area, root endodermal thickness and cell area, the cortical cell area, root metaxylem and protoxylem area and root pith area were significantly increased while cortical region thickness, metaxylem cell area and root radius reduced in ecotypes of *Justicia adhatoda* of severely dry habitats. It was concluded adaptability of *Justicia adhatoda* under drought stress was directly linked to the plasticity in root morpho-physiological and anatomical traits such as sclerification in roots epidermis, cortex and endodermis and vascular bundle that also help to conserve water and provided mechanical strength for adaptability against heterogeneous environmental conditions.

Keywords: *Justicia adhatoda, root anatomical modifications, dryness ratio, sclerification, drought tolerance*

Introduction

Water is indispensable for plant growth and development processes. Plants are more susceptible to drought stress due to changing climate. The changing climatic conditions due to various environmental constraints had intensified water deficit problems in many natural habitats (Mansoor et al., 2019). Across the world, drought stress is becoming a more significant problem that restricts plant growth and terrestrial ecosystem production (Yasir et al., 2023; Hassan et al., 2023). Climate changes occur due to atmospheric temperature fluctuations, altered rainfall pattern, irregular wind speed pattern, intensified solar radiations and differences in soil moisture contents. These environmental constraints also contribute to make soil dry, due to less soil water contents. The increasing intensity and severity of drought increase evapotranspiration in plants thereby limiting the structure and function of the terrestrial ecosystems. Ultimately, water deficit condition restricts various physiological and anatomical properties of plants including respiration, photosynthesis, and stomatal movement thus, affecting plant growth (Yang et al., 2021).

Drought is one of the key abiotic factors that affects soil and plant water status (Shao et al., 2002; El Sabagh et al., 2022). Soil water content is a crucial factor related to anthropogenic activities and influenced by various soil dryness ratio and annual rainfall pattern (Tale and Ingole, 2015; Vijayalakshmi et al., 2020). Plants respond differently in heterogeneous environments under drought stress. Plants having ability to develop various changes in their morpho-physiological and architectural patterns that help to avoid drought stress. For example, an increase root biomass and root length, reduced shoot biomass along with altered leaf area and leaf water potential has been observed (Kumar et al., 2016; Ding et al., 2021). In other studies, increased thickness of dermal tissues, sclerification of ground tissues thickness and reduced cortical and pith area and increased cortical and pith area has been reported (Mansoor et al., 2019).

Roots are main organ of a plant directly in contact with numerous biotic and abiotic factors in the soil environment. They sense environmental cues which help plants to overcome the environmental challenges. Roots induce architectural modulations to cope with heterogeneous environment for optimize growth (Naeem et al., 2023). Extensive root development is an important component of success between competitor species for water uptake and their adaptations in respective habitats. Moreover, they increase their surface area to utilize ambient water in surrounding soil that provide better resilience to cope dryness in soil (Smith and De Smet, 2012; Sofo et al., 2020). The roots' structural modulations i.e., increased epidermal and endodermal thicknesses, and sclerified steler region provides mechanical protection against heterogeneous environments. They also help plants store more water to improve plant's physiological and metabolic processes for better survival (Waseem et al., 2021; Naeem et al., 2023).

Justicia adhatoda belongs to family Acanthaceae, commonly name as vasaka. It is a perennial, small evergreen, shrub of 1.5-2 m height. It has wide distribution in India, Malaysia, and Sri Lanka, but in Pakistan, Northern latitudes of Punjab is enriched with diversity of *Justicia adhatoda*. Leaves are long, lanceolate, and dark green in color. Flowers are white with pedunculate spike (Jamwal et al., 2023). Leaves of *Justicia adhatoda* comprised of various alkaloids mainly vasicine and other organic chemical constituents including fats, essential oils, flavonoids, vitamin 'C' and phenols, showing antioxidative properties. *Justicia adhatoda* is medicinally important, commonly used in preparation of indigenous medicine. It has therapeutic use against various diseases specifically respiratory disorders (asthma, bronchitis) and gastro-intestinal disorders (diarrhea and dysentery) (Pachaiappan et al., 2021).

Morpho physiological and anatomical studies not only provide adaptive mechanism of plant species in heterogeneous environment, but also critical for evolutionary perspectives of plants with respect to changing environment. It was hypothesized that roots must have developed modulations in their physiological mechanism and roots microstructure to resist adversity of drought stress in order to maintain their root growth pattern. In this study, the main objectives were to purpose a framework to elucidate how environmental heterogeneity played a key role to evolve morpho-physiological and structural modulations in roots of *Justicia adhatoda.* It also contributes to determine viability of different ecotypes of *Justicia adhatoda* against xeric conditions. The studied modifications might help comprehend ecological fitness of *Justicia adhatoda* in native intact or degraded dry environments.

Material and methods

Selection of habitats

Nine dry habitats were selected for sampling in different districts of Punjab, Pakistan including Jehlum, Chkwal and Khushab. These habitats included Phulgran (PHU), Barakhu (BAR), Khewra (KHW), Choa Sidn Shah (CSS), Katas (KAT), Ahmedabad (AHA), Khebeki (KBL), Kallar Khar (KKR) and Jabba (JAB) where dense populations of *Justicia adhatoda* grew over wide areas. These habitats were selected on the basis of soil dryness ratio (D). The habitats with less dryness ratio were PHU, BAR and KHW. The moderately dry habitats were CSS, KAT, and AHA. The habitats with high dryness ratio were KBL, KKR and JAB.

Habitat description

Nine ecotypes (each in three replicates) of *J. adhatoda* were collected from distinct ecological regions of Punjab, Pakistan. The least dry habitats PHU(33°44'01"N, 73°12'14"E), BAR(33°42'24"N, 73°05'12"E) and KHW(32°38'53"N, 73°00'29"E) were selected to collect three ecotypes of *Justicia adhatoda* exposed to almost normal environmental conditions i.e. average annual rainfall (1220, 1024, 929 mm), soil moisture content (9.08, 6.41, 11.86%), mean annual temperature (24.5, 23.1, 24 ˚C) and dryness ratio (3.57, 4.26, 4.69). Three ecotypes are collected form moderately dry habitats i.e., CSS (32°43'19"N, 72°58'40"E), KAT (32°43'41"N, 72°56'59"E) and AHA(32°33'53"N, 72°10'17" E) (rugged dry mountainous surface) with average annual rainfall (780, 705, 685 mm), soil moisture contents (6.53, 8.84, 8.92%), mean annual temperature (18.5, 26.1, 28.5 ˚C), and dryness ratio (5.59, 6.19, 6.37). The KBL (32°37'23"N, 72°12'46"E), KKR (32°46'38"N, 72°42'18"E) and JAB (32°45'56"N, 73°00'34"E) ecotypes were selected from severely dry habitats where average annual rainfall (608, 575, 485 mm), soil moisture contents (8.52, 6.08, 6.14%), mean annual temperature (26.5, 24, 22.45 ˚C) and dryness ratio (7.17, 7.58, 8.99). Habitat details along with pictorial views of study sites are presented in *Table 1* and *Fig. 1*.

Metrological data

Metrological data i.e., annual rainfall (P), mean annual temperature (T) for different habitats were collected from PMD (Pakistan Metrological Department). Mean annual net solar radiation was estimated using daily solar radiations reported by Adnan et al. (2017). These metrological parameters are used to calculate i) Budyko-Lettau Dryness ratio (D),

ii) Air Moisture Deficit Index (MDI_{Air}) and iii) Soil Evaporative Stress Index (ESI_{soil}). Dryness ratio was calculated by using formula (D = [*R/PX L*]) devised by Budyko (1956), Air moisture deficit index (MDI_{air}) was formulated by using formulae $MDI_{Air} = (TXR) / (PXL)$ (Nathan and Sinha, 1996), while soil evaporative stress index (ESI_{soil}) was calculated by using formulae [ESI_{soil} = ((*M X L*)/ (*T XR*))]given by Yang et al. (2018) considering mean annual net radiations (R = 9855000 J m-^{2} s-^{1}) and latent heat of vaporization (L = (i.e., 2260 KJ/ mole) as constant factors. However, P = annual rainfall (mm) varied among different habitats.

Table 1. *Environment and soil physico-chemical attributes of Justicia adhatoda L. habitats*

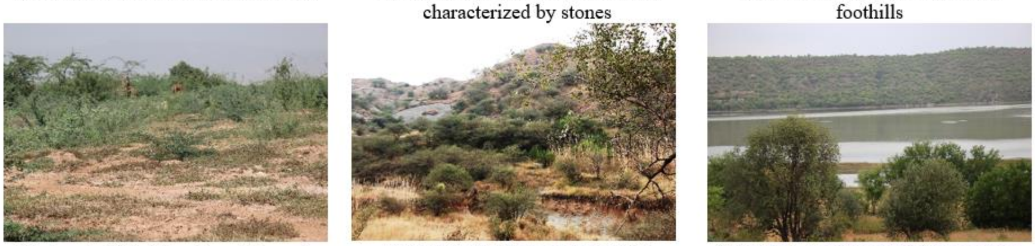
Sites	**Latitude**	**Longitude**	**Elevation**	**ARF**	**AT**	**D**	**MDI**
PHU	33°44'01"N	73°12'14"E	590	1220	24.5	3.57	87.57
BAR	33°42'24"N	73°05'12"E	544	1024	23.1	4.26	98.37
KHW	32°38'53"N	73°00'29"E	290	929	24.2	4.69	112.65
CSS	32°43'19"N	72°58'40"E	634	780	18.5	5.59	103.42
KAT	32°43'41"N	72°56'59"E	696	705	26.1	6.19	161.44
AHA	32°33'53"N	72°10'17" E	184	685	28.5	6.37	181.43
KBL	32°37'23"N	72°12'46"E	743	608	26.5	7.17	190.06
JAB	32°46'38"N	72°42'18"E	646	575	24.5	7.58	182.01
KKR	32°45'56"N	73°00'34"E	608	485	22.4	8.99	201.85
Sites	ESI	WS	RH	MC	SP	pH	ECe
PHU	0.85	8.1	43	9.08	24	7.9	0.97
BAR	0.64	9.3	24	6.41	17	8.1	0.91
KHW	1.13	8.7	35	11.86	27	6.8	0.51
CSS	0.81	5.5	27	6.53	19	8.4	0.84
KAT	0.78	8.4	37	8.83	21	7.7	1.13
AHA	0.72	4.9	37	8.91	21	7.6	1.06
KBL	0.74	4.5	39	8.52	19	7.7	1.21
KKR	0.58	7.4	34	6.08	15	9.1	0.78
JAB	0.63	7.4	52	6.14	16	8.6	0.67
Sites	OM	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	P
PHU	2.5	24.17	20.33	23.17	0.94	0.06	3.89
BAR	1.9	29.26	26.17	14.33	1.09	0.08	4.97
KHW	2.8	15.11	12.17	28.33	0.79	0.09	2.76
CSS	1.9	30.34	27.67	13.51	1.14	0.06	5.27
KAT	2.3	27.12	22.33	21.34	1.02	0.12	4.27
AHA	2.4	26.09	21.33	22.21	0.96	0.06	3.98
KBL	2.2	26.84	23.33	19.83	1.04	0.09	4.42
KKR	1.8	31.45	28.34	12.17	1.13	0.15	5.50
JAB	1.8	32.34	29.71	11.17	1.16	0.09	5.46

Means sharing same letters are statistically non-significant at $P \le 0.05$.
Sites: Phulgran (PHU), Barakhu (BAR), Khewra (KHW), Choa Sidn Shah (CSS), Katas (KAT), Ahmedabad (AHA), Khabeki Lake (KBL), Kallar kahar (KKR) and Jabba (JAB). Environmental variables: Elevation (m), Annual rainfall (ARF; mm), Average temp (AT; ^{o}C), Dryness ratio (D), MDI (Moisture deficit index), Soil evaporative index (ESI);Wind speed (WS), Relative Humidity (RH) Soil physico-chemical attributes: Soil moisture content (MC; %), Saturation percentage (SP; %), Soil pH (pH), Soil electric conductivity (ECe; dS m^{-1}) and Soil organic matter (OM, %), Sodium (Na; mg Kg^{-1}), Potassium (K; mg Kg^{-1}), Calcium (Ca^{2+}; mg Kg^{-1}), Magnesium (Mg^{2+}; mg Kg^{-1}), Chlorine (Cl^{-} mg Kg^{-1-}) and Phosphorous (P; mg Kg^{-1})

Jehlum-PHU: near mountain spring

Jehlum-BAR: Mountainous slops characterized by stones

Jehlum-KHW: Dry mountains foothills



Chakwal-CSS :flat plain with sandy dunes

Chakwal-KAT: sand-stone hills

Chakwal-AHA: muddy dunes along roadside

Khushab-KBL: Surrounding hills of hyper-saline salt lake

Khushab-KKR: near salt lake

Khushab-JAB: Steep slope, dry mountains

Figure 1. *Pictorial view of different Justicia adhatoda L. habitats. Least dry habitats: Phulgran (PHU), Barakhu (BAR), Khewra (KHW), Moderately dry habitats: Choa Sidn Shah (CSS), Katas (KAT), Ahmedabad (AHA), Severely dry habitats: Khabeki Lake (KBL), Kallar kahar (KKR) and Jabba (JAB)*

Experimental layouts

Repeated surveys were conducted to select nine habitats from different ecological sites with different soil moisture contents. Five to seven average sized plants of same age were selected from each habitats growing at their natural site under full solar radiations. Data referred to geographical location and soil characteristics of selected habitats are listed in *Table 1*.

Soil analysis

Soil samples were collected in a column upto the depth of 12 inches with the help of auger to compute soil physicochemical properties.

Soil moisture contents

Soil moisture content (MC) % in soil sample was estimated by weighing fresh weight (g) of soil and oven dried weight of soil (g) (Estefan, 2013) (*Eq.1*).

$$\text{Soil Mc }(\%) = \frac{\text{Fresh weight of soil (g)} - \text{Dry weight of soil (g)}}{\text{Fresh weight of soil (g)}} \times 100 \qquad \text{(Eq.1)}$$

Soil texture

The hydrometer method was used to record the soil texture (Gavlak et al., 2003). Soil specific textural category was determined by using USDA textural triangle method (Estefan, 2013).

Saturation percentage

Saturation paste of soil was prepared by weighing of 200 g of soil sample of soil and then mixed with distilled water (*Eq.2*).

$$\text{Saturation percentage} = \frac{\text{Amount of water used to prepare saturation paste}}{\text{Weight of soil (g)}} \times 100 \quad \text{(Eq.2)}$$

Soil pH and ECe

The filtrate was extracted using suction pump to measure soil pH, ECe, ionic contents Na^+, K^+, Ca^{+2} and Mg^{+2} and P. The soil pH and ECe were measured by using ECe and pH meter (WTW series InoLab pH/Cond 720, USA).

Soil organic matter

Organic matter contents were assessed by following (Walkley, 1947) method. A 1g of oven dried soil sample was taken in titration flask and then added potassium dichromate ($K_2Cr_2O_7$) and sulfuric acid (H_2SO_4), placed it for half an hour to homogenize the mixture. Then it was mixed dis. H_2O and ortho-phosphoric acid (H_3PO_4). Added few drops of diphenylamine as an indicator and titrated against 0.5 M Mohar's ($(NH_4)_2SO_4.FeSO_4.6H_2O$) solution until green coloration achieved. Soil organic matter was determined by using following formulas (*Eqs. 3, 4, 5*).

$$\text{Orignal Organic Carbon (\%)} = \frac{\{(\text{Volume of blank} - \text{Volume of standard})\text{x}0.3\text{xMolarity}\}}{\text{Amount of soil (g)}} \quad \text{(Eq.3)}$$

$$\text{Total organic Carbon (\%)} = 1.334 \text{ x Orignal Organic Carbon(\%)} \quad \text{(Eq.4)}$$

$$\text{Organic Matter (\%)} = 1.724 \text{ x Total Organic Carbon (\%)} \quad \text{(Eq.5)}$$

Soil ionic content

A soil saturation paste was prepared and extracted with a vacuumed filtration unit. The ionic contents sodium, potassium, and calcium (Na^+, K^+, Ca^{2+}) were analyzed by using flame photometer (Jenway, PFP-7, UK) and magnesium (Mg^{2+}) was determined by using Atomic Absorption Spectrophotometer (Hitachi Polarized Zeeman, AAS, Z-8200, Japan). Standard curves were formulated by comparing values of mineral elements mg/kg. The chloride (Cl^-) was titrated using $AgNO_3$ as standard potassium chromate ($K_2Cr_2O_4$) as indicator (Richard, 1954). Phosphorous contents were determined by preparing Barton’s reagent, values were computed by using spectrophotometer at 470 nm (UV-1100) (Hitachi-220, Japan) (Yoshida et al., 1976).

Plant material

Plants roots of *Justicia adhatoda* were collected from different dry habitats. The plant roots were packed in zipper bag and immediately brought to the laboratory to calculate morphological, biochemical, and physiological and anatomical parameters.

Morphological attributes

Natural habitats of Punjab to examine heterogeneity in their morphological attributes. Plant’s morphological attributes were observed from fresh plants sample. Dry weight of plant was determined by drying plants in oven at 65°C (REMI RDHO). The fresh and dry weights were determined by using weighing balance (SHIMADZU, Japan). The length of longest was measured by using measuring scale.

Physiological attributes

Root ionic contents were assessed by Wolf (1982) method. Dried root of *Justicia adhatoda* was placed in digestion flask and digested by adding concentrated sulphuric acid (H_2SO_4) overnight. The digestion flasks were placed on hot plate at 350-400 °C for 30 minutes until colorless fumes were produced. A 1 ml of 35% H_2O_2 was added after cooling till colorless mixture obtained. The colorless solution was then filtered by using Whatman filter paper and diluted up-to 50 ml. The filtrate is used to determine ions i.e., Na^+, Ca^{2+}, K^+, Mg^{2+} and phosphorous. The concentration of Na^+, $Ca^{2+}$and K^+ (mg/L) was recorded by using flame photometer (Jenway PFP-7, Japan). The concentration of Mg^{2+} was determined by using Atomic Absorption Spectrophotometer (Hitachi Polarized Zeeman, AAS, Z-8200, Japan).

Standards Preparation: The standard curves were made after preparing a graded series ranging from 10, 20 to 100 ppm of Na^+, K^+, and Ca^{2+}. The values of sodium (Na^+), potassium (K^+) and calcium (Ca^{2+}) were compared with standard curves to compute final concentration.

b) Phosphorus (P)

Phosphorous in root samples was analyzed by following (Yoshida et al., 1971) method. The filtrate after digestion was mixed in Barton’s reagent (Ammonium molybdate + ammonium metavanadate + nitric acid). Absorption was measured at 470 nm by using spectrophotometer (UV-1100) (Hitachi-220, Japan) and values were compared with standard curves (Jackson, 1962).

Standards Preparation: To make the standard curve, stock solution of PO_4^{3-} was prepared by dissolving monobasic phosphate (KH_2PO_4) in water. Standard series 2.5, 5, 7.5, 10, 12.5 and 15 mg/L PO_4^{3-} were prepared by adding 1, 2, 3, 4, 5 and 6 ml of PO_4^{3-}.

Anatomical attributes

a) Sample preservation: Root samples of *Justicia adhatoda* were fixed in 10% formalin solution, 50% ethanol, 5% acetic acid, and distilled water for 2 days, then preserved in 70% alcohol solution (70 ml ethanol dissolved in 30 ml distilled H_2O) to analyze anatomical attributes.

b) Sample sectioning and dehydration: Free hand sectioning was used to cut the plant samples. To dehydrate the plant sample, several grades of ethanol (30%, 50%, 70%, and 90%) were prepared.

c) Staining procedure: Sections were stained utilizing double staining procedure using two different stains (safranin and fast green). Photographs of plant sections were taken by using camera (Nikon 104, Japan) equipped with light microscope and ocular micrometer i.e., calibrated with stage micrometer.

Studied parameters: Anatomical attributes of roots of *Justicia adhatoda* were recorded. Anatomical traits of dermal tissue (epidermal thickness and area and endodermal thickness and area) ground tissue (cortex and pith) and conducting tissue (Proxylem, metaxylem and phloem) were also measured. Root radius was measured from the base of the topmost secondary root in all corrected root samples. The measurement details of these tissues are presented in *Fig. 2*.

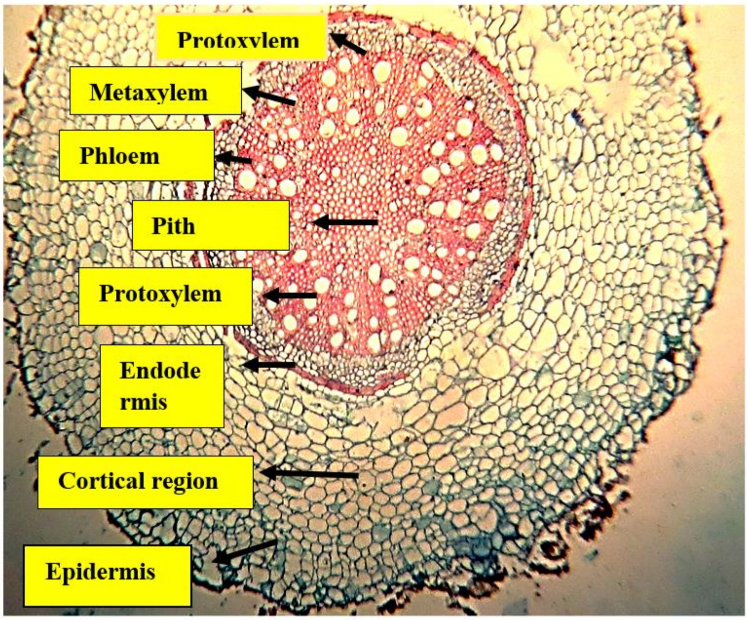


Figure 2. *Measurement details of root anatomical attributes of Justicia adhatoda L.*

Statistical analysis

Data was statistically analyzed by using COSTAT to compute LSD values. Means were computed using LSD (*p*>0.05) represented physiological and anatomical attributes. One-way analysis of variance (ANOVA) was used to test for the effect of site (Steel and Torrie, 1980). Principle component analysis was performed for association of three different environmental conditions (least dry, moderately dry, and highly dry). Correlation was drawn to correlate various physio-anatomical attributes with soil physic-chemical attributes using corplot package in R studio. The clustered heatmaps were constructed by showing association of physio-anatomical attributes of plants with soil physico-chemical traits of differently adapted population in R studio (v 4.1.2).

Results

Soil physicochemical properties

Soil physic-chemical attributes differed significantly in different habitats depending on dryness ratio in respective habitat. Among less dry habitats the minimum saturation percentage was recorded at BAR site where Budyko Lettau dryness ratio was 4.26, while among moderately dry habitats saturation percentage differed non-significantly. The maximum saturation percentage (21%) was recorded at AHA site and minimum (15.17%) was observed at CSS. But, in it was less in severely dry habitats with high Budyko Lettau dryness ratio (8.99) at KKR. Organic matter of soils at different habitats varied according to dryness of soil. The severely dry habitats showed less organic matter contents as compared to moderately and less dry habitats. The maximum (2.87%) organic contents were observed at KHW site of less dry habitats and minimum organic contents were recorded at JAB (1.77%) site located in severely dry habitats. The CSS site located in moderately dry habitat showed less (1.87%) organic contents as compared to other sites. The pH of soils of different habitats showed less variations from less acid to moderately basic (6.83-9.15) at KHW site of less dry habitats and KKR site of severely dry habitats. The ECe of soil varied differently among habitats, the maximum ECe was observed at KBL site of highly dry habitats (*Table 1*). Sodium contents varied significantly as dryness ratio increased. The maximum Na^+ and Cl^- contents (32.34 mg Kg^{-1} and 0.14 mg Kg^{-1}) was recorded at KKR site of severely dry habitats and minimum Na^+ contents (15 mg Kg^{-1}) were observed at KHW site of less dry habitats, while chloride contents were less at PHU site (0.057 mg Kg^{-1}) of less dry habitats. The potassium contents follows variations in different habitats as less dry habitats < moderately dry habitats < Severely dry habitats. But calcium contents were high at KHW site of less dry habitats, and less calcium contents were observed at KKR site of severely dry habitats. There was non-significant variation in magnesium contents of all habitats, while maximum phosphorus was recorded at KKR site. The PHU site showed low phosphorus contents (*Table 1*).

Roots morphological traits

Morphological attributes varied significantly in different habitats according to dryness ratio. Greater root fresh weight (RFW), root dry weight (RDW) was recorded at JAB site among Severely dry habitats, CSS site at moderate dry habitats and KHW site among less dry habitats. Root length (RL) were observed maximum in ecotypes of severely dry habitats (KBL and JAB) followed by ecotypes of KAT, CSS and PHU at moderately and less dry habitats, while minimum root length (RL) was recorded at KHW site of less dry habitats (*Fig. 3*).

Roots ionic contents

The root ionic contents differed significantly among habitats. Among less dry habitats the maximum root sodium (R-Na) (22.16 mg Kg^{-1}) was recorded at KHW site and minimum R-Na (15.33 mg Kg^{-1}) at PHU site, while there was less variations among CSS, KAT sites of moderately dry habitats. The maximum R-Na (24.33 mg Kg^{-1}) was recorded at JAB site of severely dry habitats. The highest root potassium (R-K) (22.33 mg Kg^{-1}) was recorded at PHU site in less dry habitats and minimum R-K contents (14 mg Kg^{-1}) were recorded at JAB site of severely dry habitat. Little variation was noted in calcium contents in less and moderately dry habitats except JAB site of severely dry habitat. The maximum value of root phosphorous (R-P) was observed at KHW site of less dry habitat, JAB sites showed less R-P contents. The root magnesium (R-Mg) varied between 12.33-19.5 mg Kg^{-1} (*Fig. 4*).

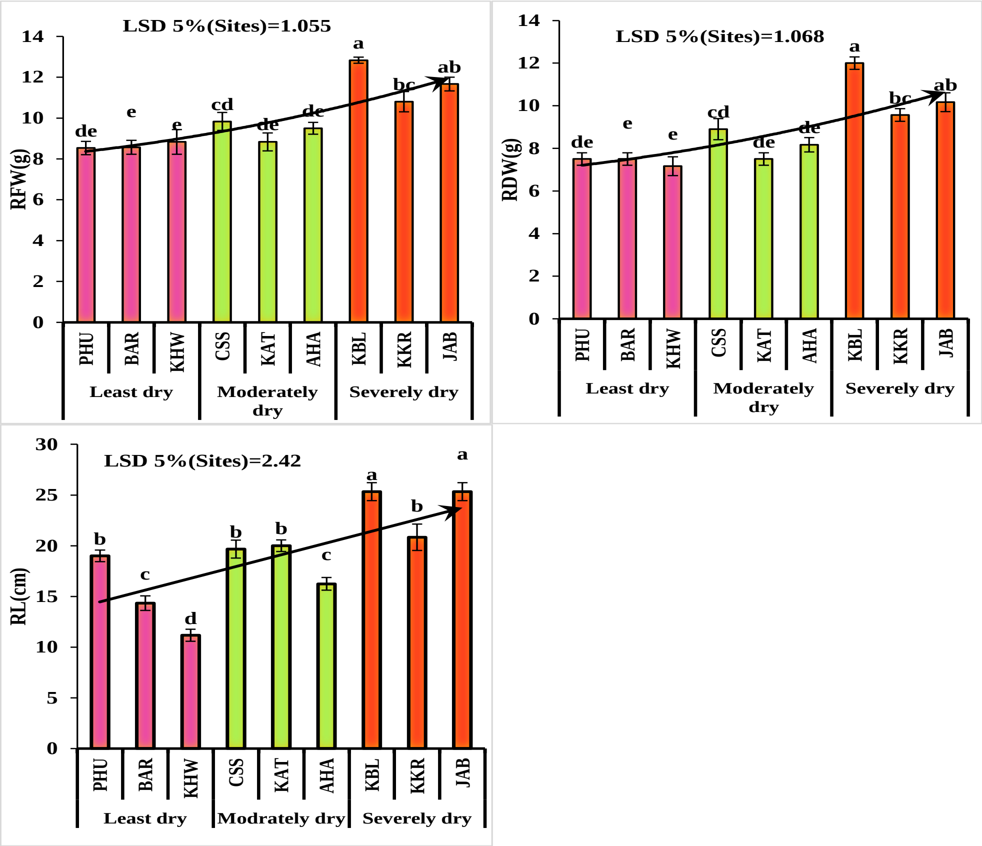


Figure 3. *Morphological attributes of Justicia adhatoda L. collected from different habitats of Punjab. Habitats were arranged according to decreasing soil MC (%) and increasing dryness ratio. Trend line was fitted to predict the possible trend of plant attributes along decreasing soil MC (%) and increasing dryness ratio. Vertical bars show SE value and same letters show values are non-significant. Least dry habitats: Phulgran (PHU), Barakhu (BAR), Khewra (KHW), Moderately dry habitats: Choa Sidn Shah (CSS), Katas (KAT), Ahmedabad (AHA), Severely dry habitats: Khabeki Lake (KBL), Kallar kahar (KKR) and Jabba (JAB). Morphological attributes: Root fresh weight (RFW), Root dry weight (RDW) and Root length (RL)*

Root anatomical attributes

Dermal tissues

Root epidermal cell area (RDA) was the maximum (264.90 µm$^{2)}$ where dryness ratio was high at KKR site of severely dry habitats. The minimum (104.30 µm^{2}) area was recorded at KHW site of less dry habitats, while ecotypes of moderately dry habitats showed greater (RDA) at CSS (204.6 µm^{2}) Root endodermal area (REA) and root endodermal thickness (RET) were recorded according to following order; Less dry habitats (BAR) < moderately dry habitats (CSS) < severely dry habitats (KKR). The moisture deficit reduced root radius (RR) in ecotypes of severely dry habitats than ecotypes of moderately and less dry habitats. The maximum root radius was observed at BAR (335.9 µm) (*Fig. 5a, 6*).

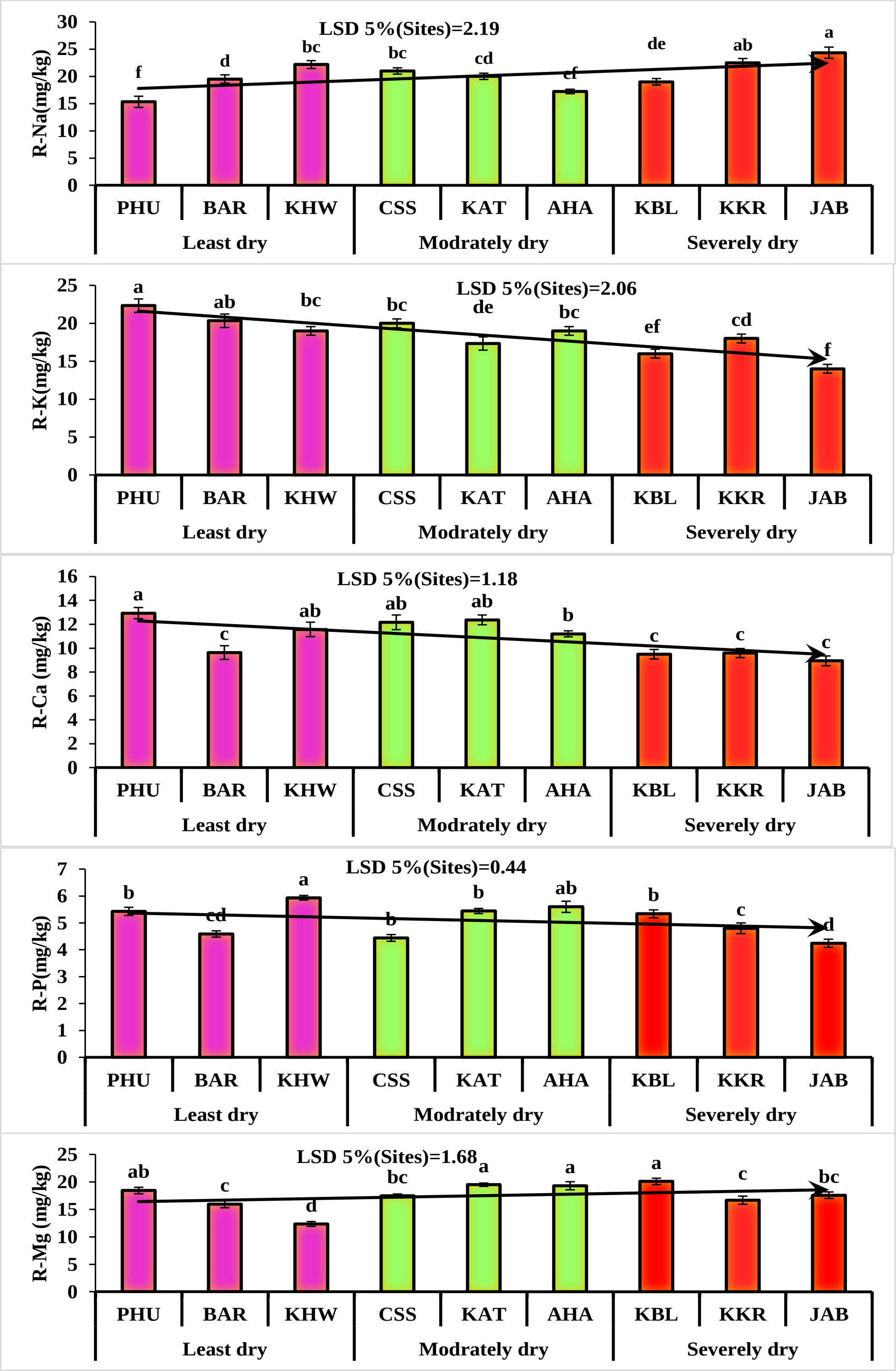


Figure 4. *Ionic contents of Justicia adhatoda L. collected from different habitats of Punjab. Habitats were arranged according to decreasing soil MC (%) and increasing dryness ratio. Trend line was fitted to predict the possible trend of plant attributes along decreasing soil MC (%) decreasing soil MC (%) and increasing dryness ratio. Least dry habitats: Phulgran (PHU), Barakhu (BAR), Khewra (KHW), Moderately dry habitats: Choa Sidn Shah (CSS), Katas (KAT), Ahmedabad (AHA), Severely dry habitats: Khabeki Lake (KBL), Kallar kahar (KKR) and Jabba (JAB). Root ionic contents: Root sodium (R-Na), Root potassium (R-K), Root calcium (R-Ca), Root phosphorus (R-P) and Root magnesium (R-Mg)*

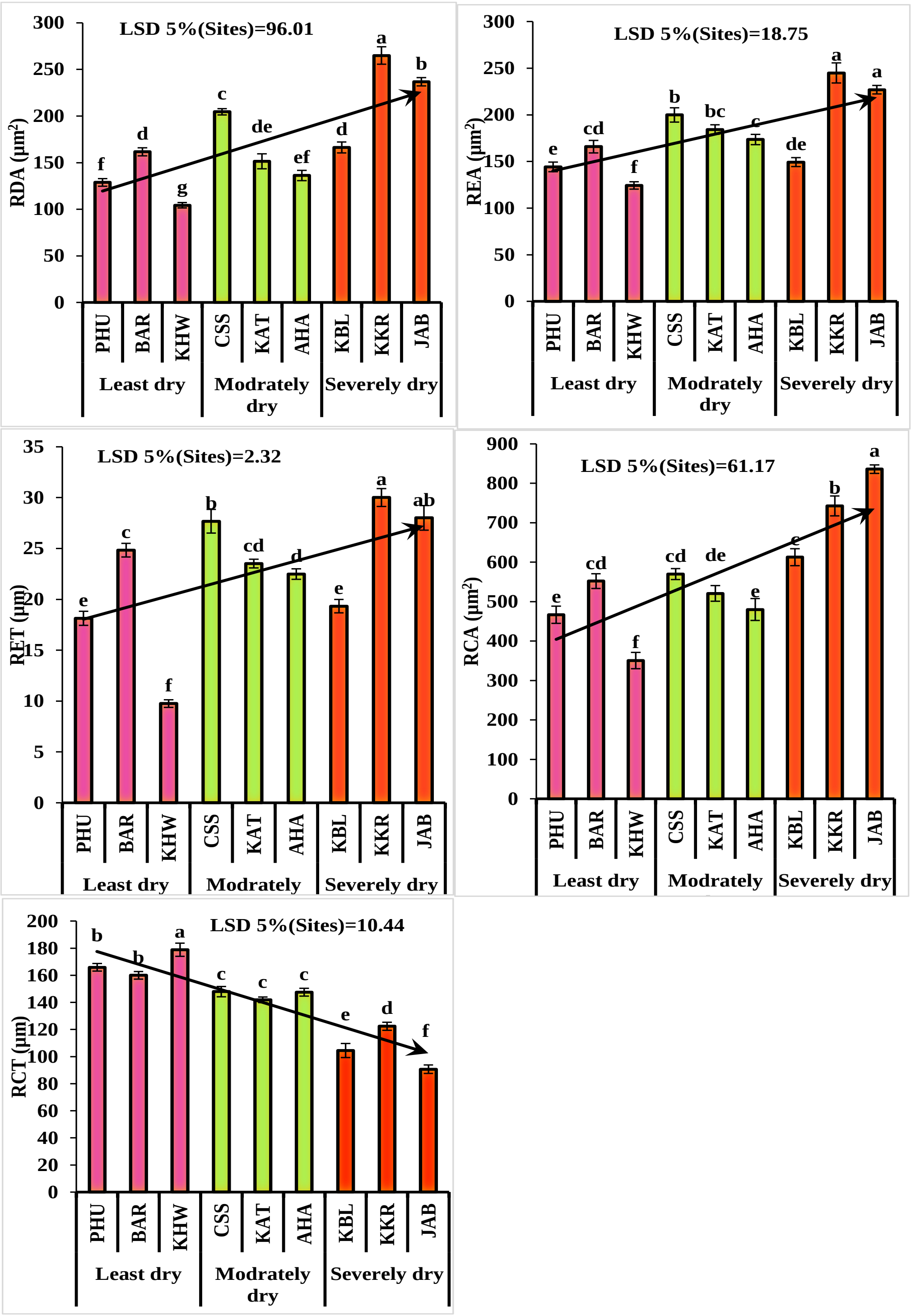


Figure 5(a). *Root anatomical attributes of Justicia adhatoda L. collected from different habitats of Punjab. Habitats are arranged according to decreasing soil MC (%) and increasing dryness ratio. Trend line was fitted to predict the possible trend of plant attributes along decreasing soil MC (%) and increasing dryness ratio. Least dry habitats: Phulgran (PHU), Barakhu (BAR), Khewra (KHW), Moderately dry habitats: Choa Sidn Shah (CSS), Katas (KAT), Ahmedabad (AHA), Severely dry habitats: Khabeki Lake (KBL), Kallar kahar (KKR) and Jabba (JAB). Root anatomical attributes: Root epidermal cell area (RDA), Root endodermal cell area (REA), Root endodermal thickness (RET), Root cortical cell area (RCA), Root cortical region thickness (RCT)*

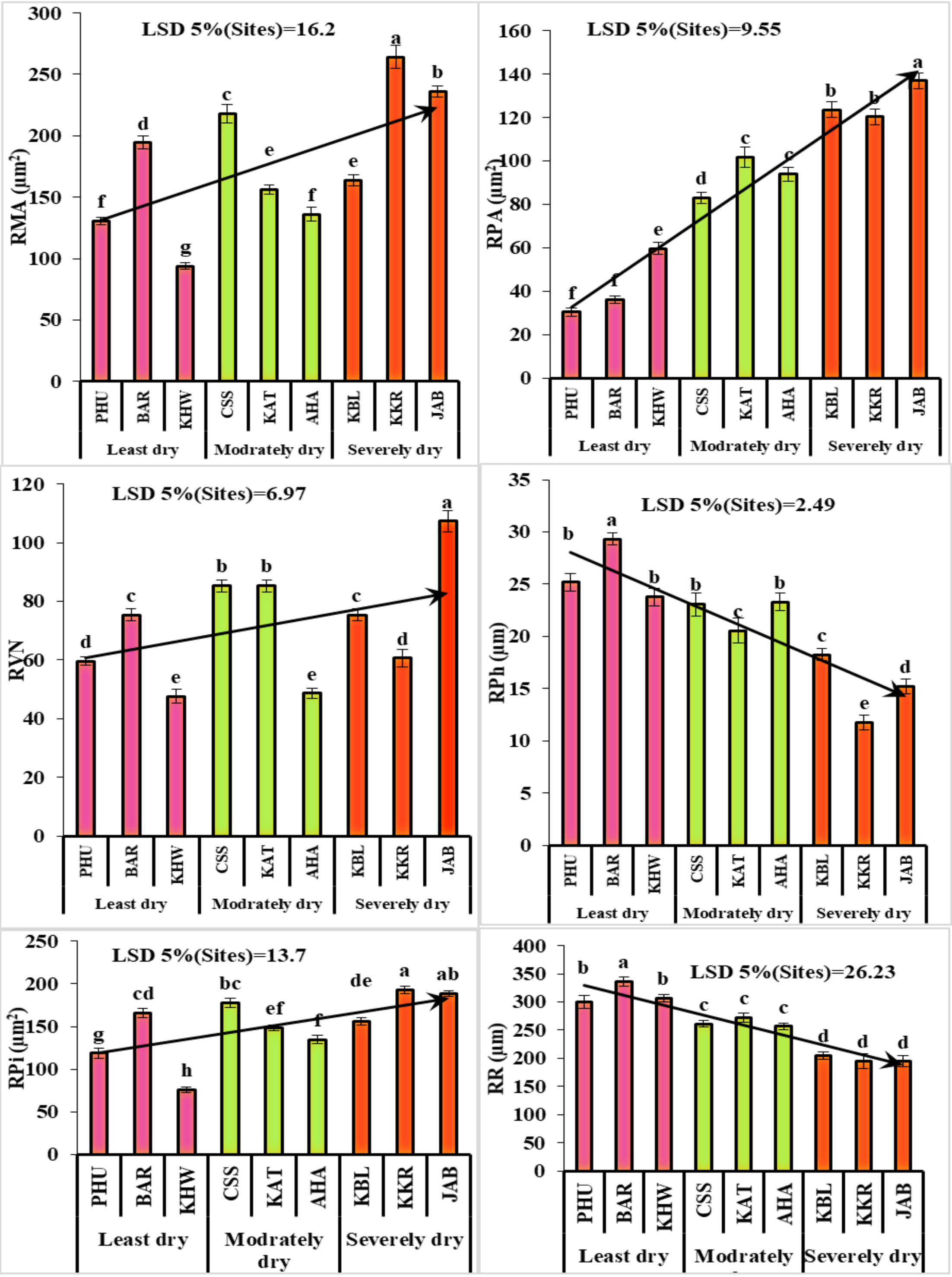
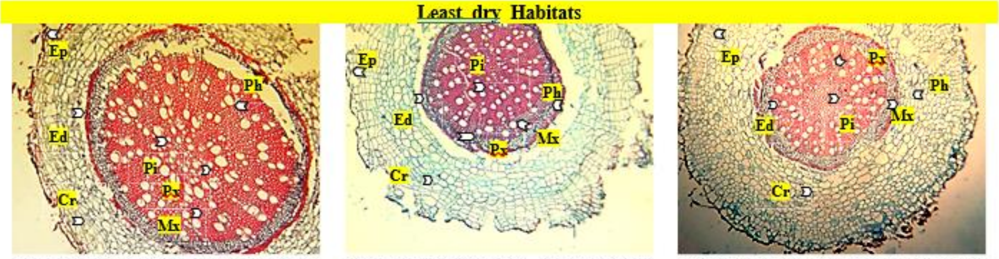


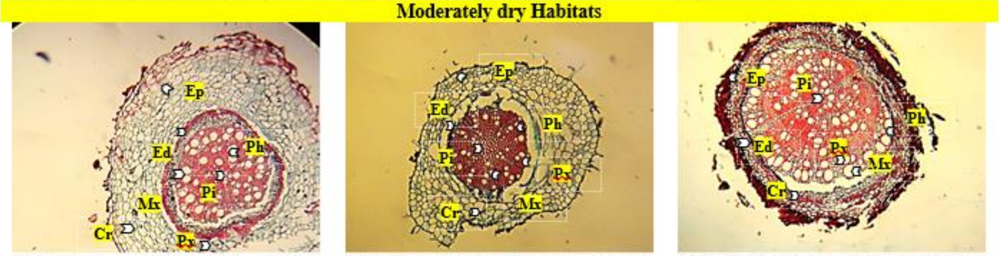
Figure 5(b). *Root anatomical attributes of Justicia adhatoda L. collected from different habitats of Punjab. Habitats are arranged according to decreasing soil MC (%) and increasing dryness ratio. Trend line was fitted to predict the possible trend of plant attributes along decreasing soil MC (%) and increasing dryness ratio. Least dry habitats: Phulgran (PHU), Barakhu (BAR), Khewra (KHW), Moderately dry habitats: Choa Sidn Shah (CSS), Katas (KAT), Ahmedabad (AHA), Severely dry habitats: Khabeki Lake (KBL), Kallar kahar (KKR) and Jabba (JAB). Root anatomical attributes: Root metaxylem cell area (RMA), Root protoxylem cell area (RPA), Root vessel number (RVN), Root Phloem thickness (RPh), Root pith area (RPi), Root radius(RR)*



PHU: Reduced epidermal (Ep) thickness; thin endodermal (Ed), enlarged pith (Pi), and cortical region (Cr) areas; lower vessel numbers.

BAR: Thin epidermis (Ep), endodermis (Ed), and metaxylem cell areas (Mx); prominent cortical region (Cr) and pith (Pi)

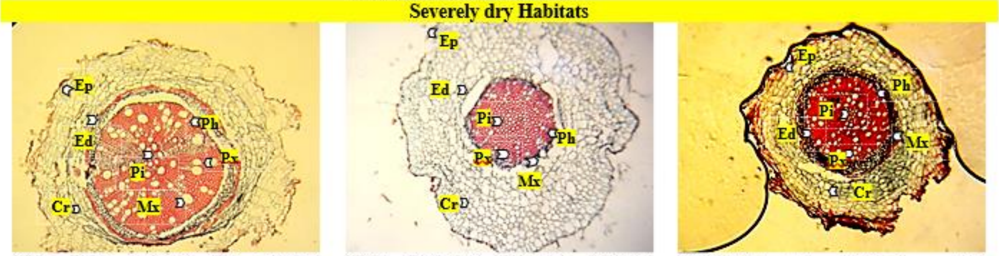
KHW: Enlarged pith (Pi), metaxylem (Mx) and cortical region (Cr) areas; reduced phloem area (Ph)



CSS: Thin endodermis (Ed), enlarged cortical region (Cr), and xylem vessels (Mx) thickness; sclerified stellar region (St)

KAT: Thin epidermis (Ep) and endodermal (Ed) cell areas, prominent and enlarged metaxylem (Mx), cortical region (Cr), phloem cells (Ph) and pith regions.

AHA: Thick epidermis (Ep) and endodermis (Ed), reduced pith (PA) and prominent cortical cells (Cr) areas



KBL: Thick epidermis (Ep), enlarged metaxylem (Mx), pith cells (Pi) areas surrounded by stele, large and constricted phloem cells (Ph)

KKR: Epidermis disrupted, increased cortical region (Cr), prominent pith area (Pi), enlarged metaxylem (Mx) surrounded by phloem (Ph)

JAB: Thick endodermis (Ed), increased pith area (Pi), constricted phloem cells (Ph), enlarged proxylem (Px).

Figure 6. *Root transverse sections of Justicia adhatoda L. collected from different habitats of Punjab, PakistanLeast dry habitats: Phulgran (PHU), Barakhu (BAR), Khewra (KHW), Moderately dry habitats: Choa Sidn Shah (CSS), Katas (KAT), Ahmedabad (AHA), Severely dry habitats: Khabeki Lake (KBL), Kallar kahar (KKR) and Jabba (JAB)*

Ground tissues

Root cortical cell area (RCA) varied significantly in different habitats. Severely dry habitats showed the maximum cortical cell area at JAB site (836.07 µm^2) and the minimum (350.60 µm^2) RCA was recorded at KHW site of less dry habitats, whereas, ecotype of moderately dry habitats at CSS site showed greater cortical cell area (569 µm^2) Cortical region thickness (CRT) varied in following order; Less dry habitats > moderately dry habitats > severely dry habitats (90.67-178.89 µm) (*Fig. 5a*, *6*). Root pith area (RPi) increased as dryness ratio increased. The maximum (192.66 µm) RPi was recorded at KKR site of severely dry habitats, while the KHW site of less dry habitat showed reduced (75.66 µm) RPi (*Fig. 5b*, *6*).

Vascular tissues

Root metaxylem area (RMA) increased significantly as dryness ration increased. Increased metaxylem area was recorded at KKR and JAB sites of severely dry habitats

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and CSS site of moderately dry habitats. Whereas variations in root protoxylem area (RPA) were observed in following order; Less dry habitats < moderately dry habitats < severely dry habitats. The highest vessel number (107) were observed at JAB site, while AHA and KHW sites showed less number (48) of vessels. Root phloem thickness (RPh) reduced as moisture deficit increased. The maximum (RPh) was observed at BAR site of less dry habitats and thin phloem was observed at KKR site of severely dry habitats (*Fig. 5b, 6*).

Multivariate analysis

Principle component analysis

Principal component analysis (PCA) revealed a significant effect of soil physico-chemical attributes specifically soil MC on the morpho- physiological and anatomical traits of *J. adhatoda* in severely dry, moderately dry, and least dry habitats (*Fig. 7*).

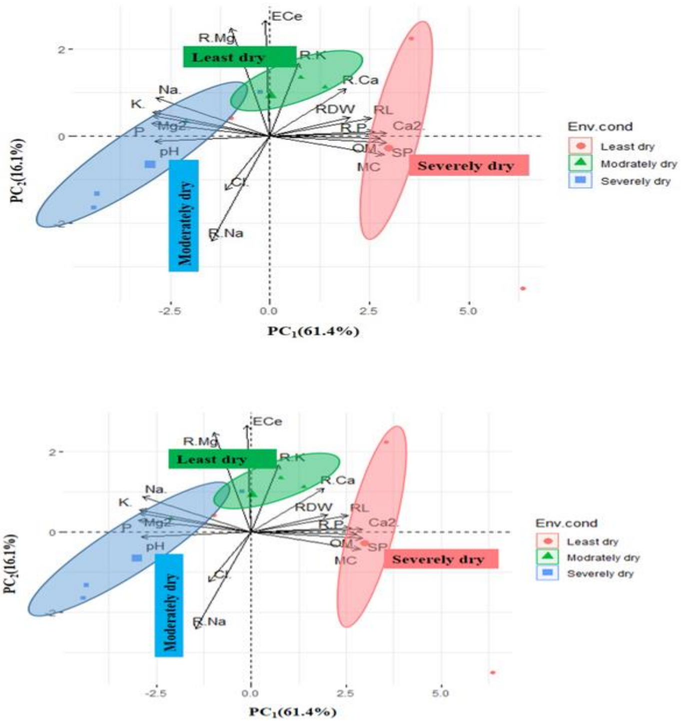

Figure 7. *Principle correspondence analysis representing (a) root morphological attributes, root ionic contents and (b) root anatomical attributes plotted against soil physicochemical attributes of Justicia adhatoda L. collected from different dry habitats. Least dry habitats [Phulgran (PHU), Barakhu (BAR), Khewra (KHW)], Moderately dry habitats [Choa Sidn Shah (CSS), Katas (KAT), Ahmedabad (AHA)], Severely dry habitats [Khabeki Lake (KBL), Kallar kahar (KKR) and Jabba (JAB)]. 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); Morphological attributes i.e., root fresh weight (RFW), root dry weight (RDW) and root length (RL). Root Ionic contents: Root sodium (R-Na), Root potassium (R-K), Root calcium (R-Ca), Root magnesium (R-Mg) and Root phosphorus (R-P). Root anatomical traits: Root epidermal cell area (RDA), root endodermal cell area (REA), root endodermal thickness (RET), root cortical cell area (RCA) and root cortical region thickness (RCT), root metaxylem area (RMA), root protoxylem area (RPX), root phloem thickness (RPT) and root pith area (RPi) root radius (RR)*

The PCAs explained 61.4% and 16.1% variability in three different dry conditions where morpho-physiological attributes were strongly related to soil physic-chemical attributes. The RDW, RL, R-Ca, R-K, R-P were the principal contributors under moderately dry environmental condition as these attributes showed positive (+2) eigenvalue (*Figure 7a*). The major components of severely dry environmental condition were R-P, MC, Ca^{2+}, OM with significantly negative eigenvalues (*Fig. 7a*). The first two PCAs explained 74.7% and 10.1% variations in three different environmental conditions among anatomical attributes with respect to soil physic-chemical attributes. The RPT, RR, RCT and soil OM, ECe and SP were the principal contributors under least dry environmental condition as these attributes showed positive (+2) eigenvalue (*Fig. 7b*). The major components of highly dry environmental condition were REA, RMA, RDA, RCA, and soil Cl^- contents with a significant reduction in positive (+4) eigenvalues (*Fig. 7b*).

Pearson's correlations

Pearson's correlation matrix between soil physicochemical and root morpho-physiological and anatomical attributes of *Justicia adhatoda* are presented in *Fig. 8*. A strong positive correlation was found between root growths attributes RFW and RDW with various soil physico-chemical attributes i.e., MC, SP, OM and Ca^{2+} and negatively with Na^+, K^+, Mg^{2+}, Cl^- and P. The root ionic contents R-Na was negatively correlated with soil MC, SP, OM and ECe and R-K, RCa, R-P, while R-K and R-Mg are non-significantly correlated with soil physic-chemical attributes (*Fig. 8a*). The correlation matrix for soil physic-chemical attributes and root anatomical traits of *Justicia adhatoda* in different environmental conditions showed strong influences. Soil MC, SP, OM and Ca^{2+} were strongly positively correlated with RCT, RPT, RR and negatively correlated with RMA, RVN, RDA, REA, RET, RPi and RCA. These anatomical features are positively correlated with soil Na^+, K^+, Mg^{2+}, Cl^- and P (*Fig. 8b*).

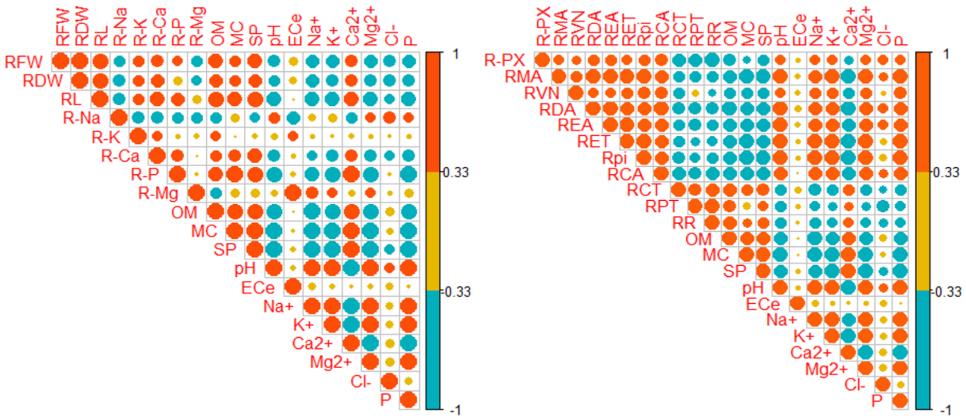

Figure 8. *Pearson's Correlation (a) root morphological attributes, root ionic contents and b) root anatomical attributes plotted against soil physicochemical attributes of Justicia adhatoda L. collected from different dry habitats. Morphological attributes i.e., root fresh weight (RFW), root dry weight (RDW) and root length (RL). Root Ionic contents: Root sodium (R-Na), Root potassium (R-K), Root calcium (R-Ca), Root magnesium (R-Mg) and Root phosphorus (R-P). Root anatomical traits: Root epidermal cell area (RDA), root endodermal cell area (REA), root endodermal thickness (RET), root cortical cell area (RCA) and root cortical region thickness (RCT), root metaxylem area (RMA), root protoxylem area (RPX), root phloem thickness (RPT) and root pith area (RPi) root radius (RR)*

Clustered heatmap

The clustered heatmap on soil [physic-chemical attributes and root morph-physiological and anatomical attributed represented a close association and grouping of root morphological parameters RFW, RDW and RL with soil MC, OM and Ca^{2+} contents at least dry habitat LD-PHU and LD-KHW (Fig.9). Among root ionic characters R-Na showed weak association with soil physic-chemical traits at least dry habitats and strong positive association with soil Na and ECe at HD-JAB habitat (*Fig. 9*) The R-K showed weak association in clustering with soil MC, Ca^{2+} at moderately dry habitat MD-KAT. The R-Mg was not associated with any soil physic-chemical attribute. Whereas, among anatomical attributes RCT, RPT showed clustering with soil MC, R-Ca, and OM at least dry habitats LD-PHU and LD-KHW. At severely dry habitats HD-JAB and HD KKR, root anatomical features i.e., RCA, RDA, REA and RMA were clustered with soil Na. whereas, RPi and RET and RVN showed clustering with soil Mg^{2+}, P and K (*Fig. 9*).

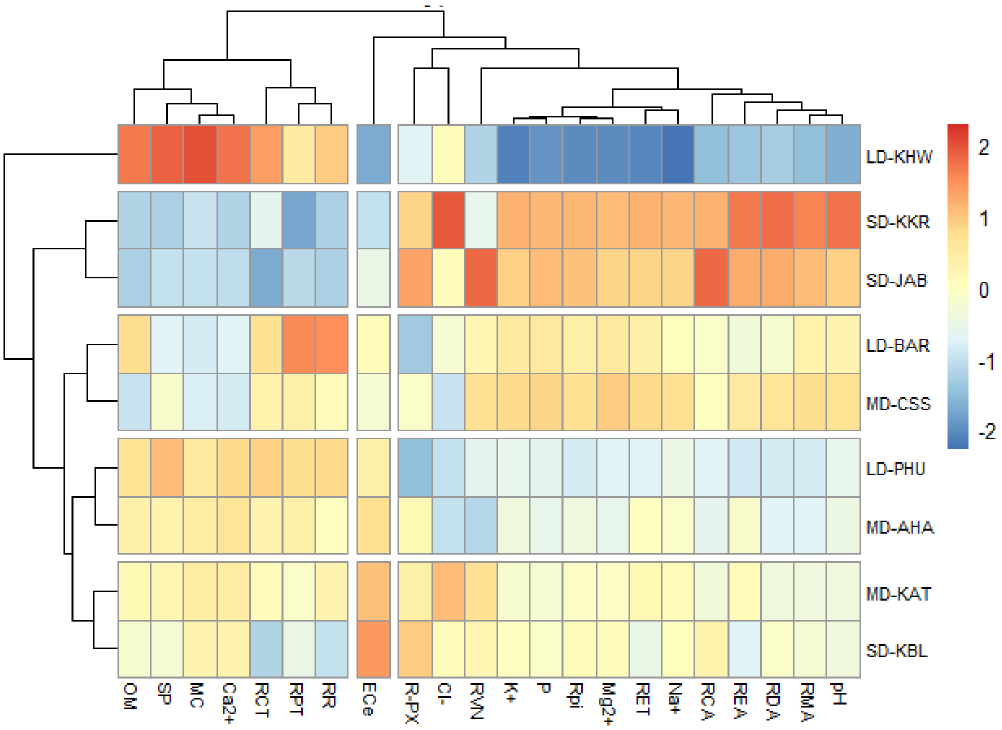

Figure 9. *Clustered Heatmaps representing (a) root morphological attributes, root ionic contents and root anatomical attributes plotted against soil physicochemical attributes of Justicia adhatoda L. collected from different dry habitats. Least dry habitats Phulgran (LD-PHU), Barakhu (LD-BAR), Khewra (LD-KHW)], Moderately dry habitats [Choa Sidn Shah (MD-CSS), Katas (MD-KAT), Ahmedabad (MD-AHA)], Severely dry habitats [Khabeki Lake (SD-KBL), Kallar kahar (SD-KKR) and Jabba (SD-JAB)]. 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); Morphological attributes i.e., root fresh weight (RFW), root dry weight (RDW) and root length (RL). Root Ionic contents: Root sodium (R-Na), Root potassium (R-K), Root calcium (R-Ca), Root magnesium (R-Mg) and Root phosphorus (R-P). Root anatomical traits: Root epidermal cell area (RDA), root endodermal cell area (REA), root endodermal thickness (RET), root cortical cell area (RCA) and root cortical region thickness (RCT), root metaxylem area (RMA), root protoxylem area (RPX), root phloem thickness (RPT) and root pith area (RPi) root radius (RR)*

Discussion

Justicia adhatoda belongs to family Acanthaceae, commonly name as vasaka (Jamwal et al., 2023), dicot, perennial plant exhibit structural (Xu et al., 2020) and functional (Berry et al., 2020) adaptations to variety of habitats against multiple abiotic stresses including drought stress due to environmental heterogeneity. The evolution of adaptive features lead to the discovery of novel tolerant ecotypes under changing xeric environmental conditions (Lone et al., 2013; Ameer et al., 2023). The present study not only points out the key adaptive morph-physiological and structural features under xeric condition. It also provides a deep insight of ecological fitness of *Justicia adhatoda* in degraded dry environments.

Abiotic stress, or drought, has grown to be the biggest problem because of a number of environmental conditions that cause water scarcity. Consequently, plants must conserve water above all else in order to survive (Islam et al., 2021; Zhang et al., 2023; Wasaya et al., 2023). It is especially critical for plants located in arid and semiarid environments to conserve water. They modify their structural (thick epidermis and cuticle, increase in cortical region, pith, and vascular bundles) and physiological attributes in order to minimize water loss (Iqbal et al., 2022; Ameer et al., 2023).

Biomass serves as a reliable measure to assess the ability of plant to tolerate stress. Resultantly, an increase in biomass production in *Justicia adhatoda* shows its substantial tolerance to water deficit. Present study indicates variations in root and shoot biomass in *Justicia adhatoda* populations growing under different dry conditions. The root fresh (RFW) and dry weight (RDW) increased in population growing in highly dry environments. The increase in root biomass helps to retain more water. Similar results was also found in *Cenchrus ciliaris* and *Heteropogon contortus* under dry conditions (Ameer et al., 2023). *J.adhatoda* showed increased root length in highly dry environments KKR and JAB. The increase in root length is an adaptive strategy of water deficit tolerant plant species to absorb and extract more water and nutrients from deep soils (Abdel Razik et al., 2021). Moreover more water extraction helps to maintain equilibrium between water consumption and CO_2 fixation in extreme dry (Mansoor et al., 2019). However, adaptations of plants against drought stress is a complex process that influence its growth and development, but, these all morphological changes account for broad distribution of *J. adhatoda* in dry habitats.

Maintenance of ionic contents in plants under dry condition is important to sustain growth and productivity. Potassium (K^+) plays significant role in maintaining cell turgidity and regulation of enzymes activity. It was reported earlier, plants growing under semi-arid and arid environmental conditions absorb higher amount of sodium along with less concentrations of potassium (K^+) and calcium ($Ca^{2+)}$ ions. These ions (K^+ and $Ca^{2+)}$ are essential for cell membrane integrity. There was a significant increase in root-Na^+ contents in *Justicia adhatoda* populations of highly dry environments KHW, BAR and JAB and a negative correlation of soil MC with root sodium contents existed (*Figure 8*) (Sun et al., 2018; Bibi et al., 2022). Present findings suggested that *J. adhatoda* ecotypes in dry habitats (KKR and JAB) possesed less K^+ and Ca^{2+} contents as compared to less and moderately dry habitats (*Fig. 4*). This might be due to more Na^+ contents in soil and groundwater affecting K^+ contents and adversely affecting enzymatic activities, cellular structure, and biosynthetic mechanism of protein (Bibi et al., 2022). Moreover, Ca^{2+} contents are positively correlated with soil moisture contents (MC) (*Fig. 8a*). The absorption of these ions (K^+, Ca^{2+}) as reported earlier in *Arabidopsis* and rice (Sun et al., 2018; Fatima et al., 2021), might counteract detrimental effects of Na^+ roots of in *Justicia* *adhatoda* thereby favors plants in their wide distribution in heterogeneous dry environments.

Plant structural features are more sensitive to environmental cues particularly drought. Their structural architecture and adaptive behavior plays a crucial role to tolerate water related environmental constraints. Therefore structural modifications are important adaptive strategies of plants in heterogeneous environment to tolerate drought stress (Sadia et al., 2021). Among root anatomical attributes, greater variations were recorded in dry habitats. Present findings revealed that increased root epidermal and endodermal cell area, epidermal and endodermal thickness, were found in roots of *J. adhatoda* ecotypes of highly dry habitats (KKR, JAB) (*Fig. 5b*). It was reported earlier, thick epidermis (Shafqat et al., 2021; Shoaib et al., 2022) help to minimize water loss. There was significant increase cortical cell area in BAR (less dry), CSS (moderately dry) and JAB (highly dry) population provide maximum space to retain more water to cope prolonged dry conditions. Moreover, larger cortical cells enhance drought resistance by reducing metabolic demands in roots, which in turn promotes root expansion and greater water uptake.

Vascular bundles (metaxylem, protoxylem and phloem) help in efficient translocation of water. Moreover, sclerification around vascular bundles (xylem and phloem) are structural adaptations against dry conditions (Bibi et al., 2022). In present study, BAR population among less dry habitats, CSS from moderately dry and KKAR from highly dry habitats had a significant increase in metaxylem vessels. Large metaxylem vessels are prominent anatomical feature for better survival in dry environments. Additionally, larger vessels facilitate water movement, nutrients, and photosynthates within the root by minimizing resistance due to their increased size. Another structural modification is increased pith cells in the BAR and KKR population of less dry and highly dry environments. Large, and loosely packed pith parenchymatous cells can store more water, playing a vital role in survival under water deficit environmental conditions (Zulfiqar et al., 2020).

Multivariate analysis also depicted positive correlation of structural attributes with soil MC contents (*Fig. 8b*). Increased root cortical cell helps to conserve water in dry habitats, sclerification around conducting tissues help better translocation of water under drought stress. Moreover, sclerification around endodermis protects ground tissues and mechanical tissues of plants against drying. These root architectural modulations provide *J. adhatoda* mechanical strength to plant tissues and also prevent collapsing of vessel elements under drought stress, thereby lowering water loss in dry conditions (Ameer et al., 2023).

Conclusion

Different habitats were selected categorized as least dry, moderately dry, and highly dry from where *Justicia adhatoda* was uprooted to explore morpho-physiological and anatomical modifications against heterogeneous environment. Plant changed their growth pattern to conserve more water and maintain ionic homeostasis for their better survival. They also modify their structure according to environmental conditions, highly dry ecotypes increased thickness of epidermis and endodermis, enlarges cortical region thickness, increased vessel number to avoid mechanical damage and conserve more water during unfavorable environmental condition. It was concluded that root adaptations in *Justicia adhatoda* enabled it to better survive in different dry environments. The increased root length, more Na^+ ions in highly dry environments (KBL, KKKR and JAB) help plants to uptake more water in order to mitigate adversity of drought stress, however, more Na^+ ions help pant in osmotic adjustment in highly dry environment. Other structural adaptive traits in *Justicia adhatoda* roots include thick epidermis, endodermis, enlarged xylem vessels and sclerified vascular bundles help to store more water and provide protection against tissue damage in water deficit environments (KBL, KKKR and JAB). Multivariate analysis depicted a strong relation of soil MC with morpho-physiological and anatomical attributes indicating these features as adaptive traits of *Justicia adhatoda* for its ecological fitness in heterogeneous environment.

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Conflict of interest. All authors have reviewed and endorsed the submission and declare that there are no competing financial or non-financial interests.

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