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PHYSIOLOGICAL AND ANATOMICAL MODIFICATIONS OF *DATURA STRAMONIUM* L. ROOTS COLLECTED FROM DRY ENVIRONMENTS

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Abstract

The effect of variable availability of soil water in natural ecosystems on structural features of roots in differently adapted populations of *Datura stramonium* was evaluated. *Datura stramonium* was collected from eight water deficit habitats. Soil physico-chemical attributes such as moisture content (%), pH, ECe, saturation percentage, organic matter (%), ionic contents i.e., monovalent ions (Na^+, K^+, and Cl^-) and divalent ions (Ca^{2+} and Mg^{2+}) were analyzed. Root physio-anatomical attributes such as ionic contents in roots (Na^+, K^+, Ca^{2+}, Mg^{2+} and P) and structural features of root such as epidermal and endodermal cell area, cortical region thickness, root radii, vessel number, root protoxylem and metaxylem, phloem area and xylem to phloem ratio were measured. The reduced availability of water significantly affected many functional and structural attributes. The most important one were reduced uptake of micronutrients and alteration in dermal, ground and conducting tissues. Population of dry habitats i.e., Jabba (JAB) and Choa Saidan Shah (CSS) tolerated the extremities of adverse environmental constraints by increasing epidermal and endodermal areas, and, cortical region thickness to protect them from mechanical injury. Additionally, decreased vessel number and increased metaxylem and protoxylem areas help to conserve more water in dry environments. In conclusion, roots of *Datura stramonium* possessed some physiological modifications by increasing uptake of sodium (Na^+) and structural changes by changing dermal and vascular tissues of root in population grown in dry habitats i.e., Choa Saidan Shah (CSS) and Jabba (JAB) site.

Key words: Water stress, Moisture content, Ion uptake, Root structural modifications, Steler region.

Abbreviations: Habitats: Pind Dadan Khan (PDK), Padhrar (PAD), University of Agriculture Faisalabad (UAF),; Chiniot (CHN), Neela Wahn (NWL), Pail (PAL), Choa Saidan Shah (CSS) and Jabba (JAB). Soil physico-chemical attributes i.e., Soil moisture content (MC%), Saturation percentage (SP), Soil pH (pH), Soil electric conductivity (ECe) and Soil organic matter (OM), Potassium (K), Calcium (Ca^{2+}), Magnesium (Mg^{2+}) and Chlorine (Cl^-).
Root ionic contents: Root sodium (RNa), Root potassium (RK), Root calcium (RCa), Root magnesium (RMg) and Root phosphorus (RPh).
Root anatomical traits: Root epidermal cell area (RDA), Root endodermal cell area (REA), Root cortical region thickness (RCT), Root pith area (RPA), Vessel number (VSN), Protoxylem area (PXA), Metaxylem area (MXA), Xylem phloem ratio XPR and Phloem area (PHA).

Introduction

Plants are subjected to a variety of environmental stress during growth and development while growing under both natural and agricultural conditions. Water deficit is considered as an inevitable environmental factor that drastically affects plant growth and finally hampers plant biomass. It is the dynamic environmental factor and depends on changes in temperature and rainfall patterns (Seleiman *et al.*, 2021). In several countries, including Pakistan, water scarcity has become an alarming condition for plant growth and productivity particularly for those plant species having low resistance to water stress. Pakistan's environment is diverse, with a variety of landscapes including plains, sand dunes, Rocky Mountains, forests, and coastal regions. Low rainfall in various arid and mountainous region of Pakistan causes severe soil water deficit. Therefore, the drought-tolerance in plant species is the key adaptive strategy to deal with water scarcity problems in natural habitats (Abd Elbar *et al.*, 2019; Ahmad *et al.*, 2019) where, environmental constraints i.e., moisture and temperature are major cause of moisture deficit. In addition to these environmental constraints, soil physicochemical characteristics along moisture deficit gradient adversely affected physio-anatomical attributes of plants. It might have a direct impact on plant water content, which then has a negative impact on plant productivity and survival (Jia *et al.*, 2021).

Roots serve as barrier between the plant and various abiotic factors prevailing in the soil environment. They respond to fluctuations in environment and enable plants to tolerate environmental constraints. The roots are the primary organ for plant water intake and are critical for crop production, specifically under limited water supply. The root length is considered as a functional attribute that better defines a root's ability to explore deeper layers of soil in response to water stress (Lynch, 2011). Generally, drought affects root characterized by above ground changes in cuticle and epidermal characteristics. Irrespective of morphological and structural adaptations physiological traits related to water potential and ionic homeostasis are also associated with plant water status (Smith & De Smet, 2012).

Plant species evolve certain adaptive traits in response to abiotic stress at the population level, ensuring their survival in their native habitat. The morpho-anatomical, physiological, and biochemical features may alter the structure and function of particular ecotype of native habitat (Yamori *et al.*, 2014). These modifications help them to overcome adverse environmental constraints. Differentially adapted populations of plants evolve distinct changes in anatomical characteristics specifically sclerified hypodermal, endodermal and cortical region, larger xylem vessels, thicker endodermis for radial movement of water in roots. These are principal strategies to alleviate the adversity of less water availability, also provide high tolerance in plants against water stress (Fatima *et al.*, 2021).

Datura stramonium (Jimsoon weed) is an important member of the Solanaceae family (Das *et al.*, 2012). It is an annual C_3 wild plant species has worldwide distribution in Central America, Asia and Africa. This medicinal plant is major source of alkaloids i.e., tropane, tannin and phytic acids. It has long been use in various biological activities such as anti-asthmatic, antibacterial, antifungal, anti-inflammatory, antioxidant against chronic diseases. *D. stramonium* are likely to respond to the changing highly dynamic environment due to its diverse phenological and physiological processes. In order to plan control measures due to environmental changes brought on by upcoming climate changes. The lack of scientific literature describing how environmental changes, specifically reduced water availability, affect structural and physiological activities of *Datura stramonium*, is a significant problem. In this context, it was hypothesized that soil moisture contents varied in dry habitats imposed structural and functional changes in roots of *Datura stramonium* L. for to tolerate severity of drought condition. The objectives of this comprehensive study were to (i) examine the effect of drought on ionic imbalance and homeostasis in different populations of *Datura stramonium* (ii) understand how physio-anatomical characteristics of *Datura stramonium* are affected by varying soil moisture levels; and (iii) evaluate the effect of soil moisture deficit on root anatomical attributes of *Datura stramonium* by determining the dryness ratio in their respective environment.

Materials and Methods

Study surveys and sample collections: A survey of different habitats of Punjab was conducted to explore the adaptive components of *Datura stramonium*. Plant and soil samples of *Datura stramonium* were collected from eight distinct habitats i.e., Pindadan Khan (PDK), Padhrar (PAD), University of Agriculture Faisalabad (UAF), Chiniot (CHN), Katas (KAT), Neela Wahn (NWL), Pail (PAL), Sheikhpura (SKP), Choasidn Shah (CSS) and Jabba (JAB). Metrological data i.e., precipitation (mm), Average minimum and maximum temperature ($^{\circ}$C) were record from Metrological substations of their respective district. Coordinates (latitude and longitude) and altitude of selected habitats were determined by Global positioning system (GPS, model: Garmin E-Trex 20) (Fig. 1).

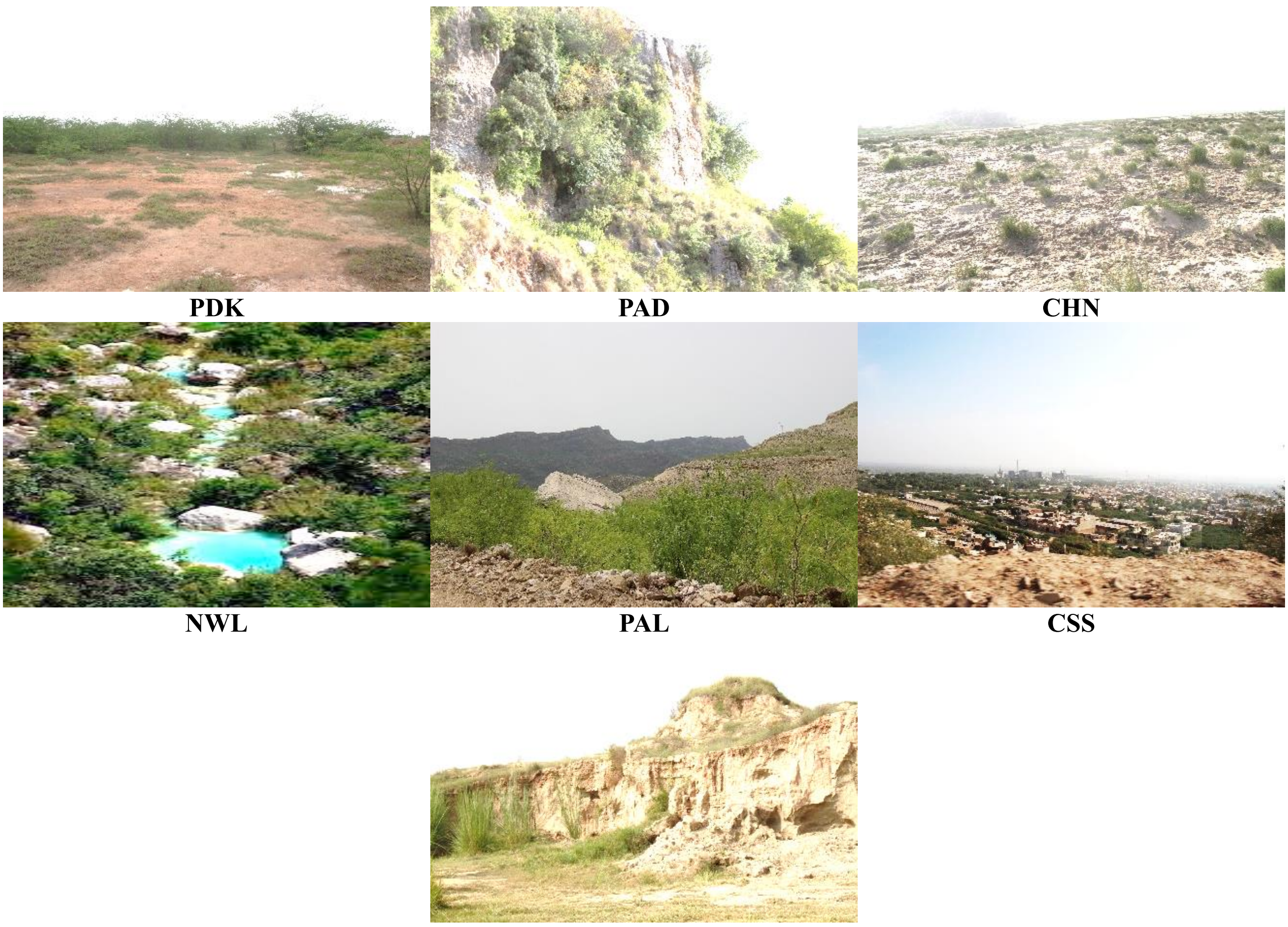


Fig. 1. Pictorial view of different *Datura stramonium* habitats.
Sites: Pindadan Khan (PDK), Padhrar (PAD), University of Agriculture Faisalabad (UAF), Chiniot (CHN), Pail (PAL), Choa Sidn Shah (CSS) and Jabba (JAB).

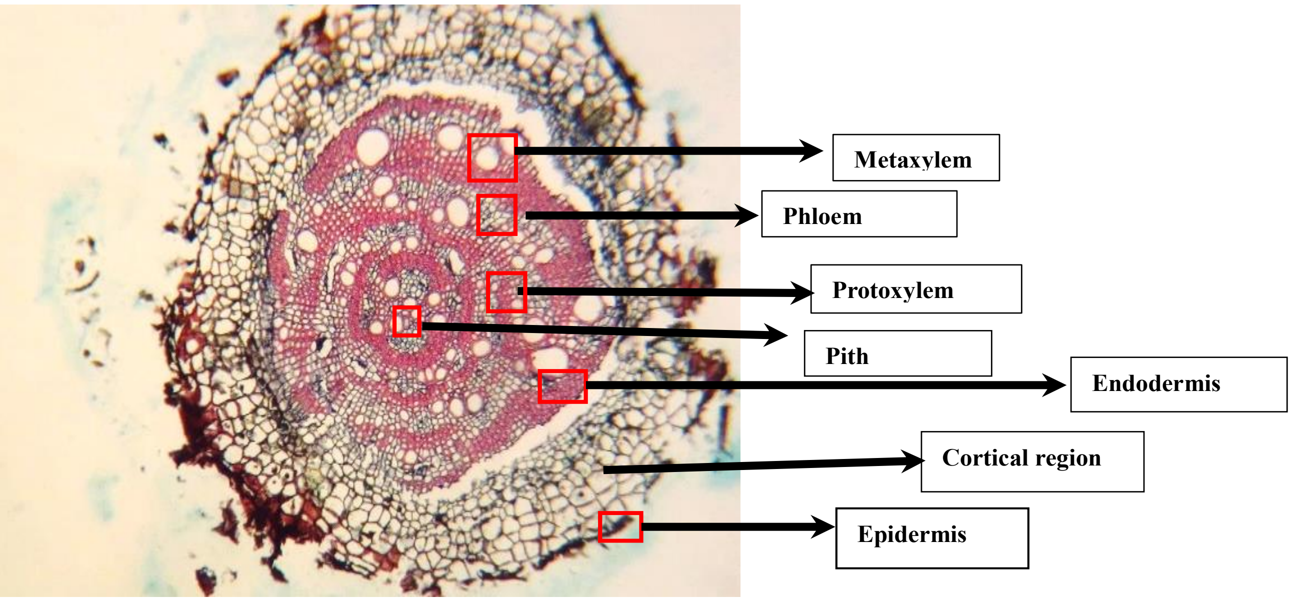


Fig. 2. Measurement details of root anatomical attributes of *Datura stramonium*.

Soil physicochemical traits: The rhizospheric soil of different rooting zone was collected from each sampling site to analyze various physicochemical attributes. A 200 g of oven-dried soil sample was taken to prepare saturation paste to determine soil saturation percentage (SP), pH, ECe and ionic contents in soil. The soil water was extracted by using suction pump to determine pH and ECe of soil by using pH/EC meter (pH/Cond 720, WTW series InoLab, USA). A flame photometer (PFP-7, Jenway, UK) was used to determine ionic contents in soil (Na^+, K^+ and Ca^{2+}). The magnesium (Mg^{2+}) contents in soil were determined by using atomic absorption spectrophotometer (AAnalyst 300, PerkinElmer, USA). Standardized titration method by using $AgNO_3$ (as standard), potassium chromate (K_2 Cr O_4) solution (as indicator) for estimation of chloride ion (Cl) (Richard, 1954).

Plant ionic content: Plant ionic contents were determined (Wolf, 1982). Fresh plant leaf samples of (0.5g) were taken and soaked overnight in 5ml H_2SO_4 in a digestion flask. The samples were heated on hot plate (at 350°C). Then H_2O_2 was added to decolorize the solution. Monovalent (Na^+ and K^+) and divalent ion (Ca^{2+}) were determined by using flame photometer (Model 410, Sherwood Scientific Ltd., Cambridge, UK). The magnesium (Mg) contents were assessed by using atomic absorption spectrophotometer (AAnalyst 300, PerkinElmer, USA). The standard curves were generated after preparing a graded series of standards ranging from 10, 20 to 100 ppm of Na^+, K^+, and Ca^{2+}. Phosphorous (P) was analyzed by following Yoshida (Yoshida *et al.*, 1971) method. Barton's reagent was prepared. The filtrate was mixed in Barton's reagent then compute absorption at 470 nm by using spectrophotometer (UV-1100) as described by Jackson (Jackson, 1962). The values were compared with standard series to calculate actual P values.

Anatomical attributes: To explore anatomical attributes, the thickest roots were collected by digging the soil by using soil auger. Plant material was kept for 3 days in formalin acetic alcohol (FAA) (5% formaldehyde, 10% acetic acid, 50% ethanol and 35% distilled H_2O) (Fig. 2). To preserve for longer time, root samples were socked in acetic alcohol solution. Free hand sectioning was done by using razor blade. Root samples were stained by standard double staining method following Ruzin (Ruzin, 1999). Safranin and fast green were used to stain the root tissues. Permanent slides were prepared and micrographs of stained slides were taken on a stereomicroscope (Nikon 104) equipped with a digital camera (Nikon FDX-35).

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 traits. One-way analysis of variance (ANOVA) was used to test for the effect of site (Steel & Torrie, 1980). Redundancy analysis (RDA) in CANOCO for Windows (v. 4.5) was used to determine the environmental influences on the plant physio-anatomical traits. Then, RDA triplots were created by putting the soil's physical characteristics (factor 1) from various sites (factors 2) impacting the attributes of the plants that were observed (factor 3). The response of plant attributes against soil MC gradients was evaluated by drawing response curves. 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 physio-chemical traits: Soil MC (%) of the University of Agriculture Faisalabad (UAF) site was the highest (12%), while that of the Jaba (JAB) was the lowest (7.7%). Soil pH of the JAB site (8.4) was the maximum. The higher ECe was found at JAB (1.8 dS m^{-1}) and smaller ECe was recorded at (0.6 dS m^{-1}) Pind Dadan Kan PDK site. Soil Na (34.2 mg L^{-1}), Mg^{2+} (1.18 mg L^{-1}), and organic matter % (1.05%) were significantly higher in the JAB habitat. Soil K^+ (23.3 mg L^{-1}) and soil Cl^- (3.67

mg L^{-1}) were also high at JAB site and Ca^{+2} (29.5 mg L^{-1}) were the highest in at UAF site (Table 1).

Root ionic contents: Root sodium (RNa) were higher than other ionic contents (K^+, Ca^{2+}, Mg^{2+}). Potassium (RK) concentrations in root varied as it was reduced with increased dryness ratio. The maximum concentration of potassium (RK) was observed in Pind Dadan Khan (PAD) ecotype (19.7mg/kg, 15.2mg/kg), while the minimum values of RK were recorded at JAB population (9.9mg/kg, 7 mg/kg) (Table 2). The relative concentration of magnesium and phosphorous ion (RMg and RPh) in roots varied significantly. The magnesium contents (RMg) in root differed significantly with constantly reducing moisture contents in rhizospheric soil of respective habitat. But the maximum root phosphorous (RPh) was found in the population of PDK (0.44 mg/kg) (Table 2).

Root anatomical traits: Root epidermal cell area (RDA) significantly increased along increasing moisture deficit. Larger RDA was observed in Jaba (JAB) ecotype (229.14 µm) and smaller RDA was observed at Pind Dadan Khan (PDK) ecotype (97.08 µm) (Fig. 3). Endodermis is the characteristic features of roots that were substantially increased with increasing soil moisture deficit. The maximum endodermal area (REA) was observed at JAB (682.5 µm) but the ecotype of University of Agriculture, Faisalabad (UAF) had smaller endodermal area (272.96 µm). Remarkable variation in thickness of cortical region was observed as dryness ratio increased. A decrease from (165.41 µm) at Neela Wahn (NWL) to (68.11 µm) at Pail (PAL) site was observed in the cortical region thickness. Root radius varied significantly, where the larger root radii was recorded in JAB population followed by Chiniot (CHN) and NWL (390.3 µm), while smaller root radii (205.16 µm) were observed at PDK site. The root pith area substantially increased with increasing moisture deficit of the habitats, which was the maximum in the JAB population (188 µm), while the minimum was seen in the Padhrar (PAD) population (92.3 µm). The vessel number was significantly high in JAB (65) populations of *D. stramonium*. Plants collected from less water deficit habitat i.e. PDK habitats of *D. stramonium* exhibited the minimum vessel number (12). Metaxylem and protoxylem areas were the maximum (3573.1 µm and 430.44 µm) in JAB population of *D. stramonium,* while PDK population showed the smaller (173 µm and 59.6 µm) vessel area (both metaxylem and protoxylem). The maximum phloem area was recorded in the JAB population (7609 μm^2) and the minimum was seen in the PAD population (1138 μm^2) (Table 2).

Redundancy analysis*:* RDA demonstrated an association of soil physicochemical attributes with root ionic contents of *D. stramonium* of different habitats. At Choa Saidan Shah (CSS) and Pail (PAL) habitats, RMg and RPh were strongly related to soil Mg, Cl, Na, and ECe. The RNa was associated with soil OM, pH and K, while soil Ca, SP and MC at University of Agriculture, Faisalabad (UAF) habitat were correlated with RCa (root calcium). At Padhrar (PAD) and Pind Dadan Khan (PDK) habitats, root potassium (RK) was not influenced by any soil physico-chemical attributes (Fig. 4). Among anatomical features, RPh (root phloem area), RDA (root epidermal cell area), RCA (root cortical cell area) showed a strong association with soil SP, Ca, MC in Neela Whan (NWL) and UAF habitats. The population from Jaba (JAB) and CSS exhibited a correlation with soil Cl, Na and K that show association with RMA (root metaxylem area). Soil ECe OM, pH and P showed less association with PXA (protoxylem area), RVN (root vessel number), RXP (root xylem to phloem ratio) at Chiniot (CHN) site (Fig. 4).

Table 1. Environment and soil physico-chemical attributes of different habitats of the Punjab, Pakistan.

Sites	Latitude	Longitude	ALT	ARF	AT	ST	MC	SP
PDK	32°35'17"N	73°02'36"E	204	801	25.2	SL	11.0 ± 0.2^{b}	24 ± 0.5^{ab}
PAD	32°39'21"N	72°29'28"E	807	790	26	SCL	11.2 ± 0.3^{ab}	24.3 ± 0.8^{ab}
UAF	31°25'39"N	73°04'32"E	185	740	26.5	SL	12 ± 0.2^{a}	25 ± 0.6^{a}
CHN	31°43'56"N	72°58'53"E	185	620	32.5	SL	8.6 ± 0.2^{c}	23 ± 0.5^{b}
NWL	32°39'55"N	72°37'70"E	833	588	25.5	SCL	8.2 ± 0.2^{cd}	20.1 ± 0.6^{c}
PAL	32°37'54"N	72°27'29"E	807	570	26	SL	7.7 ± 0.4^{d}	18.1 ± 0.41^{d}
CSS	32°43'19"N	72°58'40"E	634	519	18.5	SL	6.5 ± 0.14^{e}	18 ± 0.57^{d}
JAB	32°45'56"N	73°00'34"E	608	508	22.45	SL	5.7 ± 0.14^{e}	17.3 ± 0.8^{d}
	pH	**ECe**	**OM**	**Na^+**	**K^+**	**Ca^{2+}**	**Mg^{2+}**	**Cl^-**
PDK	6.4 ± 0.26^{c}	0.6 ± 0.03^{f}	0.36 ± 0.03^{d}	19.1 ± 0.7^{d}	10.2 ± 0.6^{d}	27.2 ± 0.4^{bc}	0.78 ± 0.02^{d}	1.86 ± 0.04^{e}
PAD	6.7 ± 0.11^{c}	0.8 ± 0.02^{e}	0.39 ± 0.02^{d}	20.1 ± 0.4^{d}	10.7 ± 0.4^{d}	28.2 ± 0.6^{ab}	0.8 ± 0.017^{d}	1.89 ± 0.07^{e}
UAF	6.8 ± 0.3^{c}	0.83 ± 0.03^{e}	0.64 ± 0.06^{c}	18.3 ± 0.6^{d}	9.6 ± 0.5^{d}	29.5 ± 0.9^{a}	0.82 ± 0.02^{d}	1.90 ± 0.02^{e}
CHN	7.7 ± 0.08^{b}	1.02 ± 0.02^{d}	0.71 ± 0.05^{c}	24.1 ± 0.4^{c}	14.7 ± 0.60^{c}	26.3 ± 0.4^{c}	0.86 ± 0.03^{cd}	2.09 ± 0.06^{d}
NWL	7.8 ± 0.09^{b}	1.2 ± 0.03^{c}	0.9 ± 0.05^{b}	27.8 ± 0.7^{b}	18.3 ± 0.4^{b}	24 ± 0.6^{d}	0.92 ± 0.02^{c}	2.56 ± 0.09^{c}
PAL	7.79 ± 0.06^{b}	1.3 ± 0.01^{b}	1.04 ± 0.02^{a}	29.8 ± 0.7^{b}	19.4 ± 0.42^{b}	24.5 ± 0.8^{d}	0.94 ± 0.04^{c}	3.13 ± 0.05^{b}
CSS	8.3 ± 0.09^{a}	1.7 ± 0.02^{a}	1.13 ± 0.02^{a}	33.9 ± 1.01^{a}	22.3 ± 0.7^{a}	18.5 ± 0.5^{e}	1.07 ± 0.03^{b}	3.54 ± 0.05^{a}
JAB	8.4 ± 0.1^{a}	1.8 ± 0.02^{a}	1.05 ± 0.04^{a}	34.2 ± 0.6^{a}	23.3 ± 0.6^{a}	17.3 ± 0.4^{e}	1.18 ± 0.01^{a}	3.67 ± 0.04^{a}

Means sharing same letters are statistically non-significant at $p \leq 0.05$

Sites: Pindadan Khan (PDK), Padhrar (PAD), University of Agriculture Faisalabad (UAF), Chiniot (CHN), Neela Wahn (NWL), Pail (PAL), Choa Sidn Shah (CSS) and Jabba (JAB). Environmental variables: Altitude (ALT; m), Annual rainfall (ARF; mm), Average temp (AT; °C), Soil Texture (ST); 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, %), Potassium (K; mg Kg^{-1}), Calcium (Ca^{2+}; mg Kg^{-1}), Magnesium (Mg^{2+}; mg Kg^{-1}) and Chlorine (Cl^- mg Kg^{-1}). Soil texture: Sandy loam (SL), Sandy clayey loam (SCL)

Table 2. Root Physio-anatomical attributes of *Datura stramonium* of different habitats of the Punjab, Pakistan.

Sites	RNa (mg Kg^{-1})	RK (mg Kg^{-1})	RCa (mg Kg^{-1})	RMg (mg Kg^{-1})	RPh (mg Kg^{-1})
PDK	16.2 ± 0.6^{d}	18.9 ± 0.92^{a}	14.2 ± 0.6^{ab}	1.83 ± 0.05^{b}	2.72 ± 0.117^{a}
PAD	17.2 ± 0.61^{d}	19.7 ± 0.72^{a}	15.2 ± 0.9^{a}	1.97 ± 0.04^{ab}	2.3 ± 0.113^{ab}
UAF	17.7 ± 0.4^{cd}	17.9 ± 0.44^{a}	14.3 ± 0.88^{ab}	2.04 ± 0.06^{a}	2.19 ± 0.116^{b}
CHN	20.33± 0.7^{bc}	13.7 ± 1.59^{b}	12.5 ± 0.76^{bc}	1.59 ± 0.04^{c}	1.69 ± 0.196^{c}
NWL	22.2 ± 0.7^{ab}	14.2 ± 0.60^{b}	12.1 ± 0.6^{c}	1.54 ± 0.06^{c}	1.45 ± 0.179^{cd}
PAL	23.5 ± 1.5^{a}	14.5 ± 0.57^{b}	11 ± 0.2^{cd}	1.82 ± 0.07^{b}	1.22 ± 0.168^{d}
CSS	23.7 ± 1.64^{a}	12.7 ± 0.88^{b}	9.7 ± 0.44^{d}	1.83 ± 0.05^{b}	0.63 ± 0.173^{e}
JAB	24.2 ± 0.44^{a}	9.9 ± 0.60^{c}	7 ± 0.57^{e}	1.97 ± 0.04^{ab}	0.44 ± 0.124^{e}

Sites	RDA (µm^2)	REA (µm^2)	RCT(µm)	RRA (µm^2)	RPA (µm^2)
PDK	97.08 ± 8.76^{d}	335.61 ± 21.30^{de}	82.01 ± 6.0^{de}	205.16 ± 21.2^{e}	96.9 ± 5.7^{f}
PAD	107.63 ± 6.69^{cd}	372.27 ± 15.66^{ef}	125.1 ± 4.8^{b}	285.23 ± 18.6^{cd}	92.3 ± 2.8^{f}
UAF	111.30 ± 9.87^{cd}	272.96 ± 12.26^{de}	101.47 ± 9.7^{cd}	236.8 ± 20.5^{de}	113 ± 9.1^{e}
CHN	105.08 ± 7.33^{d}	295.41 ± 11.08^{d}	134.83 ± 8.4^{b}	349.6 ± 15.7^{ab}	128 ± 4.9^{de}
NWL	125.63 ± 8.58^{cd}	427.94 ± 13.01^{c}	165.41 ± 8.4^{a}	390.3 ± 7.89^{a}	138 ± 4.9^{cd}
PAL	135.27 ± 7.87^{c}	456.82 ± 10.79^{c}	68.11 ± 7.7^{e}	240.13 ± 17.0^{de}	154 ± 6.1^{bc}
CSS	182.15 ± 7.55^{b}	624.12 ± 17.52^{b}	120.93 ± 3.6^{bc}	278.56 ± 19.5^{cd}	170 ± 4.1^{b}
JAB	229.14 ± 17.1^{a}	682.51 ± 15.62^{a}	127.88 ± 7.3^{b}	330.26 ± 21.2^{bc}	188 ± 3.5^{a}

Sites	VSN ($root^{-1}$)	PXA (µm^2)	MXA (µm^2)	XPR	PHA (µm^2)
PDK	12 ± 1.2^{f}	59.6 ± 12.4^{e}	173 ± 55.47^{g}	0.15 ± 0.018^{f}	1141 ± 51.9^{d}
PAD	16 ± 0.9^{f}	81.8 ± 15.8^{de}	236.8 ± 36.47^{fg}	0.2 ± 0.019^{ef}	1138 ± 102.7^{d}
UAF	27 ± 1.45^{e}	136.7 ± 14.7^{cd}	565.6 ± 25.67^{ef}	0.2 ± 0.016^{e}	2599 ± 189.5^{c}
CHN	31 ± 0.6^{d}	167.9 ± 28.7^{c}	1266 ± 146.54^{e}	0.3 ± 0.007^{d}	3811 ± 501.5^{bc}
NWL	39 ± 1.8^{c}	239.7 ± 12.4^{b}	837.9 ± 132.46^{d}	0.2 ± 0.020^{ef}	4608 ± 751.8^{b}
PAL	41 ± 2.08^{c}	230.05 ± 26.2^{b}	1644.07 ± 155.22^{c}	0.52 ± 0.006^{a}	3143 ± 178.4^{c}
CSS	52 ± 1.5^{b}	277.8 ± 16.5^{b}	2759.2 ± 161.74^{b}	0.42 ± 0.016^{c}	6779 ± 512.4^{a}
JAB	65 ± 1.4^{a}	430.44 ± 18.3^{a}	3573.1 ± 168.40^{a}	0.5 ± 0.019^{b}	7609 ± 477.9^{a}

Means sharing same letters are statistically non-significant at $p\leq0.05$

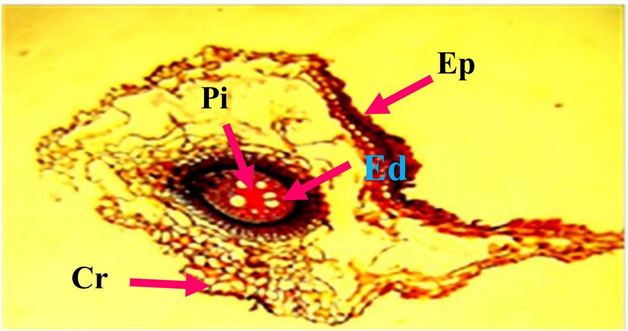
Sites: Pind Dadan khan (PDK), Padhrar (PAD), University of Agriculture Faisalabad (UAF), Chiniot (CHN), Pail (PAL), Choa Sidn Shah (CSS) and Jabba (JAB). Root Ionic contents: Root sodium (RNa), Root potassium (RK), Root calcium (RCa), Root magnesium (RMg) and Root phosphorus (RPh). Root anatomical traits: Root epidermal cell area (RDA), Root endodermal cell area (REA), Root cortical region thickness (RCT), Root pith area (RPA), Vessel number (VSN), Protoxylem area (PXA), Metaxylem area (MXA), Xylem phloem ratio XPR and Phloem area (PHA)

Response curves against soil moisture gradients: Response curves representing root ionic contents i.e., RNa showed a negative trend line against increasing soil MC, while RCa, RK, RPh and RMg were plotted with positive trend line along soil MC gradient. Root anatomical attributes, RCT, RDA, PXA, RRA, RXP, RVN, REA, RMA, RPA and RPh were negatively slopped against increasing soil moisture (MC). The RRA and PXA showed co-linearity with increasing soil moisture contents (Fig. 5).

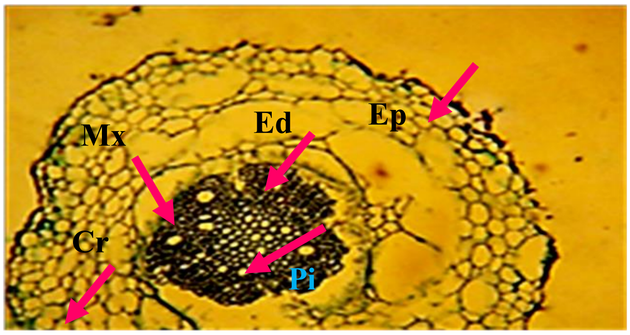
Clustered heatmap: Clustered heatmap representing grouping of root ionic content of *Datura stramonium* and soil physicochemical attributes are shown in (Fig. 6). Cluster 1 in heat map revealed the grouping of soil physicochemical traits i.e., soil Ca and SP with RCa at UAF, PDK and PAD habitats while, RPh and RK were closely related to MC. Cluster 2 of heat map indicated the grouping among soil OM, Mg, Cl, Na, P with RNa (root sodium) at CSS and JAB sites (Fig. 6a). Among plant anatomical and soil physio-chemical attributes cluster 1 was the largest cluster and divided into three sub-clusters. Sub cluster 1 displayed root anatomical attributes i.e., RXP closely associated with soil pH and OM at CSS and JAB sites. Sub cluster 2 depicted strong influence of soil Na, K, Cl on REA, RPA and RVN at CSS and JAB habitats, while population of PDK, PAD and UAF were less affected by any soil physico-chemical attribute. Sub cluster 3 depicted low influence of soil P, ECe and Mg on RMA, RDA, RPh at PAL CHN and NWL habitats (Fig. 6b).

Discussions

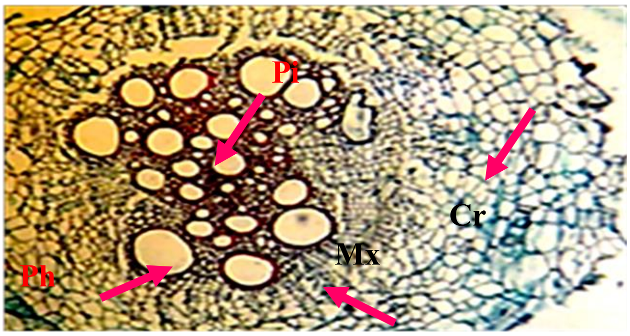
Water scarcity shows hazardous effects on plants such as growth inhibition, ionic imbalance, suppression of enzymatic activities and alteration of microstructural features of roots. Plant use defensive mechanism against these environmental stresses for effective utilization of energy in their metabolic pathways (Lahiri *et al.*, 2021). In the present study, physiological and anatomical traits of *Datura stramonium* ecotypes under water deficit were examined. Populations of *Datura stramonium* collected from different habitats exhibited remarkable variations according to varying soil dryness ratio. This study found that, JABA environment was drier as compared to other habitats. Natural habitats vary according to dryness ratio depending on topographical, metrological and soil physico-chemical attributes that showed a significant impact on adaptive strategies of native populations (Bazihizina *et al.*, 2009).



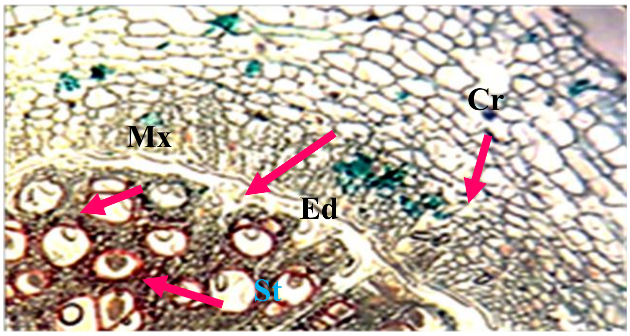
PDK: Reduced epidermal (Ep); endodermal (Ed), pith (Pi), and cortical region (Cr) areas; lower vessel numbers



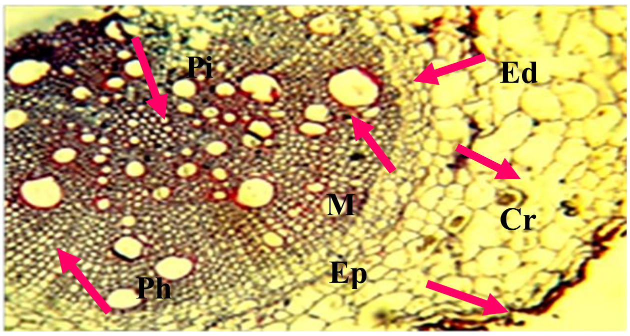
PAD: Thin epidermis (Ep), endodermis (Ed), and metaxylem cell areas (Mx); prominent cortical region (Cr) and pith (Pi)



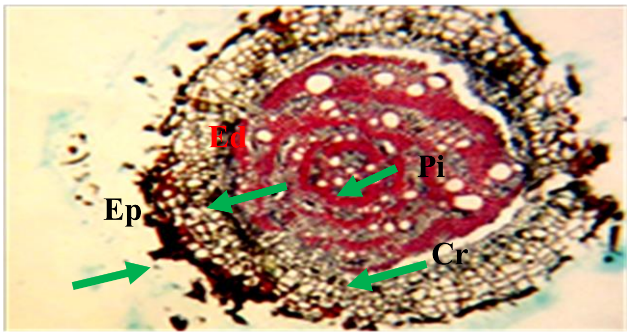
UAF: Enlarged pith (Pi), metaxylem (Mx) and cortical region (Cr) areas; reduced phloem area (Ph)



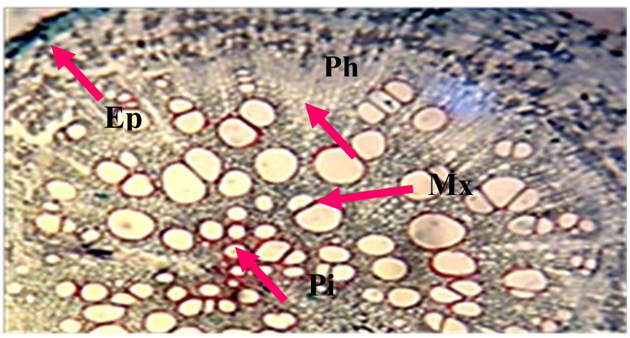
CHN: Thin endodermis (Ed), enlarged cortical region (Cr), and xylem vessels (Mx) thickness; sclerified stellar region (St)



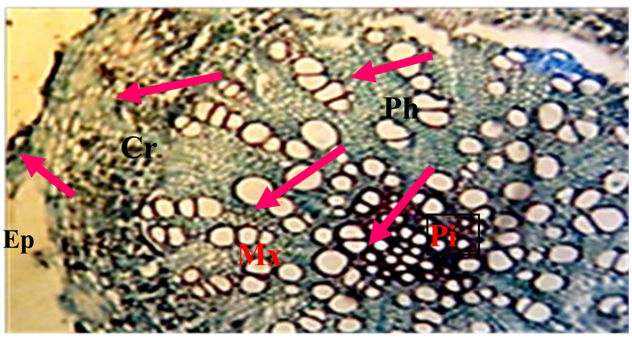
NWL: Thin epidermis (Ep) and endodermal (Ed) cell areas, prominent and enlarged metaxylem (Mx), cortical region (Cr), phloem cells (Ph) and pith regions.



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



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



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

Fig. 3. Root transverse sections of *Datura stramonium* collected from different habitats of Punjab, Pakistan. Sites: Pindadan Khan (PDK), Padhrar (PAD), University of Agriculture Faisalabad (UAF), Chiniot (CHN), Pail (PAL), Choasidn Shah (CSS) and Jabba (JAB).

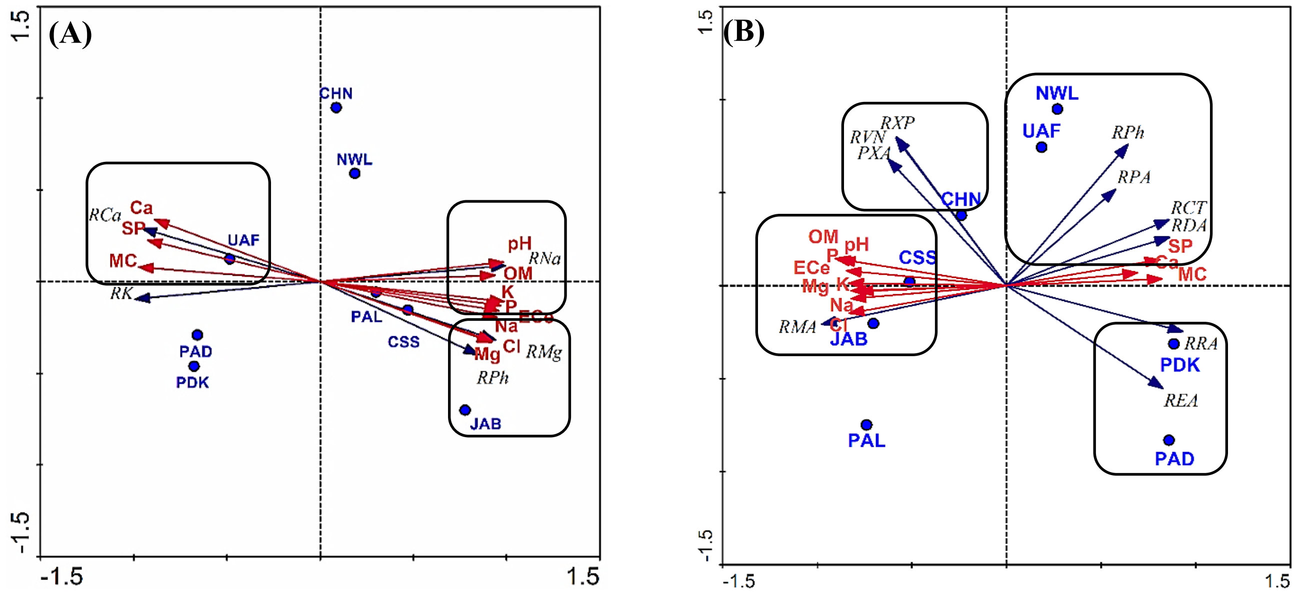


Fig. 4. RDA tri-plot representing root ionic contents (a) and root anatomical attributes (b) plotted against soil physicochemical attributes of *Datura stramonium* collected from different habitats.

Sites: Pindadan khan (PDK), Padhrar (PAD), University of Agriculture Faisalabad (UAF), Chiniot (CHN), Pail (PAL), Choasidn Shah (CSS) 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); **Root Ionic contents:** Root sodium (RNa), Root potassium (RK), Root calcium (RCa), Root magnesium (RMg) and Root phosphorus (RPh). **Root anatomical traits:** Root epidermal cell area (RDA), Root endodermal cell area (REA), Root cortical region thickness (RCT), Root pith area (RPA), Vessel number (VSN), Protoxylem area (PXA), Metaxylem area (MXA), Xylem phloem ratio XPR and Phloem area (PHA)

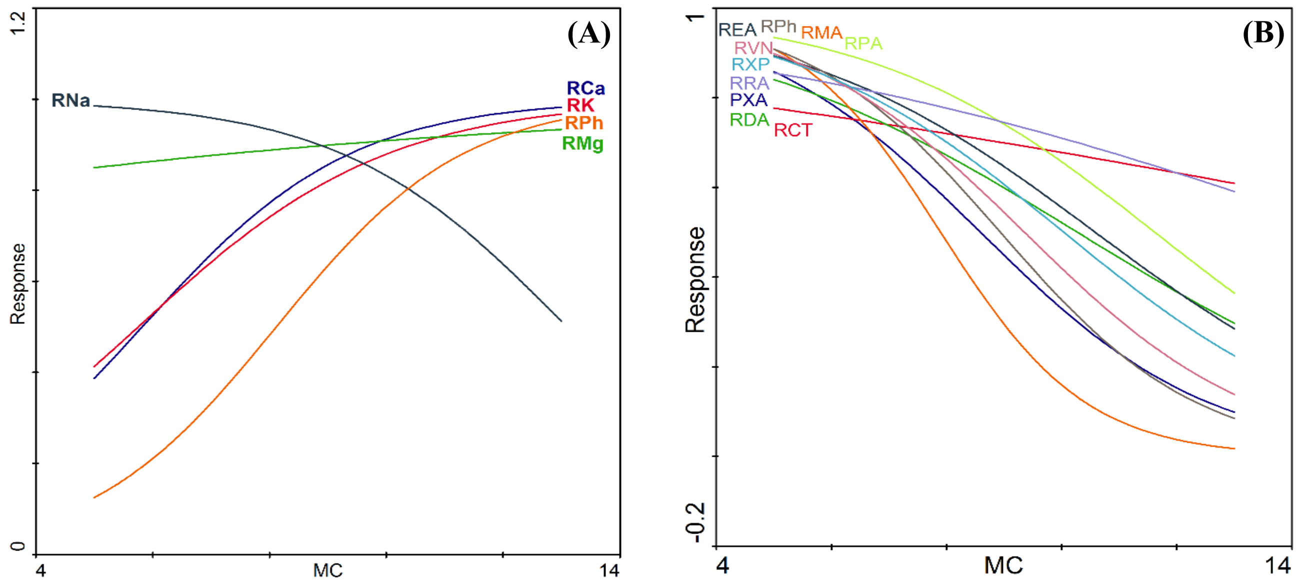


Fig. 5. Response Curves (RC) for a) root ionic contents and b) root anatomical attributes plotted against increasing moisture content (left to right) of rhizospheric soil of *Datura stramonium* collected from different habitats.

Root ionic contents: Root sodium (RNa), Root potassium (RK), Root calcium (RCa), Root magnesium (RMg) and Root phosphorus (RPh). **Root anatomical traits:** Root epidermal cell area (RDA), Root endodermal cell area (REA), Root cortical region thickness (RCT), Root pith area (RPA), Vessel number (VSN), Protoxylem area (PXA), Metaxylem area (MXA), Xylem phloem ratio XPR and Phloem area (PHA).

Water stress significantly influences plant water status. It might lower leaf water potential (Chaves *et al.*, 2002; Kaleem & Hameed, 2021) and is associated with reduced water and ion uptake by roots as reported earlier in *Solanum tuberosum* (Hu & Schmidhalter, 2005). Low water contents in soil slows down translocation of ions through roots. While root Na^+ concentration reduced in response to a severe water shortage that is independent of sodium content in leaf that shows a positive association with root's relative water content (Fayyaz *et al.*, 2013). Contrary to these findings, present findings revealed the RNa increased in *D. stramonium* along increasing moisture deficit. It takes a less selective system than other cations to absorb Na^+ at the root/soil interface. Multiple types of channels can allow sodium to enter plant cells, hence increasing Na^+ uptake in some plants under water deficit conditions (Ali & Abdur, 2017; Assaha *et al.*, 2017).

An important inorganic plant solute, potassium ion (K^+), helps reduce the osmotic potential in stellar region of roots which promotes the development of turgor pressure and the movement of solutes through the xylem (Almeida *et al.*, 2017; Fatima *et al.*, 2021). In order to maintain turgor at low leaf water potentials and reduce the adverse effects of drought, potassium concentration may help in osmotic adjustment (Kumar *et al.*, 2017). Present findings suggested that water scarcity reduced the uptake of RK and RCa contents, where the maximum root calcium contents were observed in PDK and PAD ecotypes. The reduced RK was possibly due to Na^+ and K^+ co-transporters that restricted uptake of potassium ion through roots under water stress (Kumar *et al.*, 2017).

Anatomical features and strategic behavior of plants are considered as adaptive traits to avoid water stress. It was reported that the roots of plants modify themselves for better survival by increasing the size and density of parenchyma cells, sclerification, thicker epidermis and endodermis (Chen *et al.*, 2016; Carrizo *et al.*, 2021; Zia *et al.*, 2021). Similar findings were reported by (Ahmad *et al.*, 2016). These anatomical modifications provide efficient water uptake and storage space, which are essential for survival (Dossa *et al.*, 2017). Recent studies showed that the root epidermal cell area (RDA), root endodermal cell area (REA) and root cortical region thickness (RCT) substantially increased with increasing water stress. The maximum RDA, REA and RET were observed at JAB site, while PDK showed the minimum RDA, REA and RCT. These anatomical changes are vital to reduce water loss from outer surface of root and stelar region (Ahmad *et al.*, 2016).

It was found the environmental abiotic stress in different habitats altered the structure of plants. For example, the radius of root increased due to enlargement of cell size of parenchymatous, and, pith and conducting tissues that provide better resilience against mechanical injury (Nawaz *et al.*, 2013). Presently, NWL ecotype of *D. stramonium* showed maximum root radius and minimum pith area was observed at JAB site. The thick sclerified vascular bundles i.e., xylem and phloem help to prevent outflow of water and solutes from roots (Rahat, 2019; Sarwar *et al.*, 2022). Current findings revealed the increased cell size of conducting tissues (xylem and phloem) and parenchyma of *D. stramonium* at moisture deficit habitats CSS and JAB. These structural changes are dynamic strategies for water conservation and are achieved either by reducing water loss through the plant surface or by conserving water inside the plant body and by storing water inside the plant body (Al-Tawaha *et al.*, 2017; Khan *et al.*, 2017). It was also studied in roots of desert species, low water availability adversely affected root stelar region i.e., metaxylem, phloem, and pith region which is the indication of inhibited growth under extreme water stress (Barzegargolchini *et al.*, 2017; Huang *et al.*, 2018).

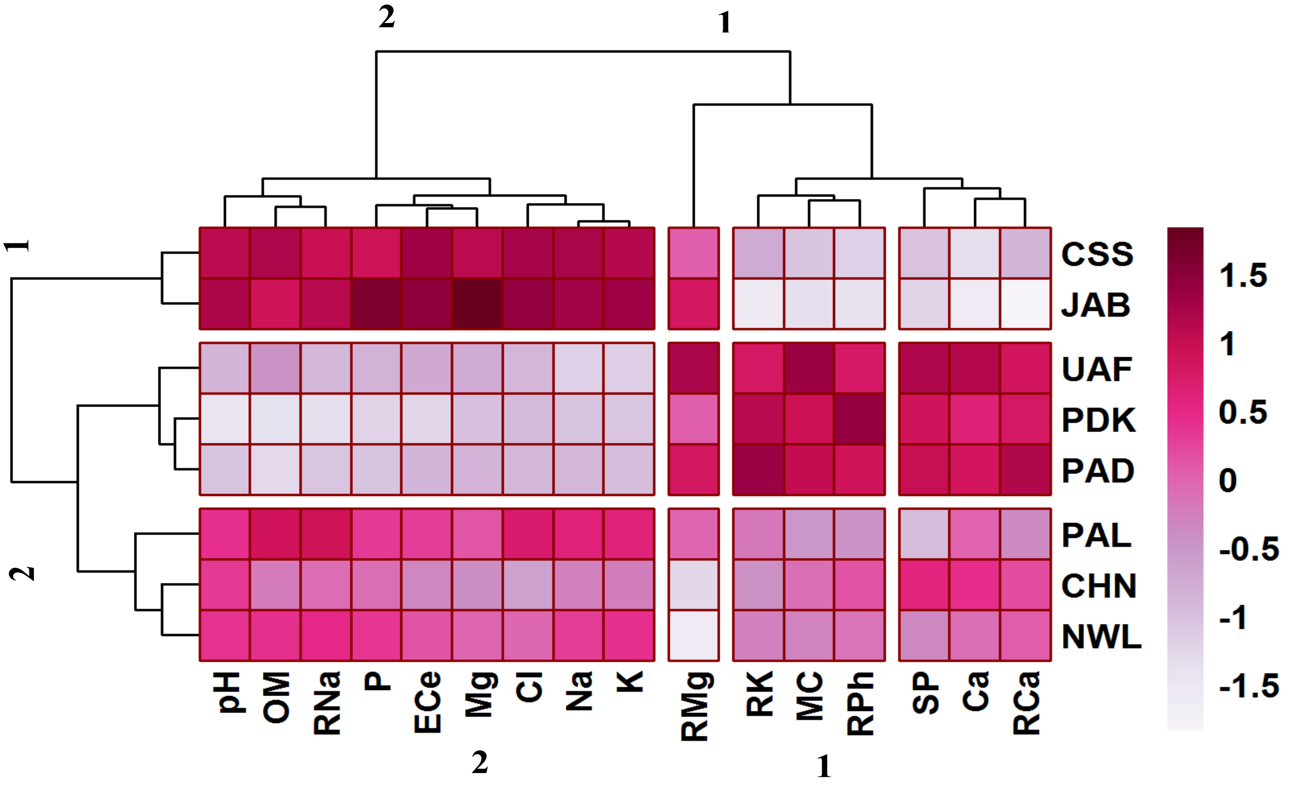


Fig. 6. Clustered heat map for a) root ionic contents and b) root anatomical attributes with soil physico-chemical attributes of rhizospheric soil of *Datura stramonium* collected from different habitats.

Root Ionic contents: Root sodium (RNa), Root potassium (RK), Root calcium (RCa), Root magnesium (RMg) and Root phosphorus (RPh). **Root anatomical traits:** Root epidermal cell area (RDA), Root endodermal cell area (REA), Root cortical region thickness (RCT), Root pith area (RPA), Vessel number (VSN), Protoxylem area (PXA), Metaxylem area (MXA), Xylem phloem ratio XPR and Phloem area (PHA)

The RDA triplot showed that the concentration of sodium in soil affected RNa at PAL site (Figure 4). The response curves indicated that RNa contents respond negatively with increasing moisture deficit condition. It was also revealed that RCa and RK contents in *D. stramonium* were strongly associated with calcium contents in soil. Moreover, these ions are positively correlated with soil moisture contents suggesting that more water contents in soil help more uptake of calcium and potassium contents under water limited environments (Iqbal *et al.*, 2022). Moreover, the effect of rhizospheric soil of different habitats strongly affected the structural attributes of root of *D. stramonium*. It was also represented by a negative response curve of anatomical attributes of roots with respect to soil MC. These findings suggest a differential effect of water stress on structure and function of *D. stramonium* and its adaptive response to tolerate water scarcity (Kumar *et al.*, 2017; Mansoor *et al.*, 2019).

Conclusion

Root are most sensitive organs that respond differently under water stress. It alters the structural and functional attributes of roots in order to withstand water scarcity. Population of *Datura stramonium* collected from heterogenic environments behaved differently in different habitats. Ecotypes of extreme water deficit environment possessed some adaptive features for better survival. *Datura stramonium* ecotype from CSS and JAB, the driest habitat, comprised of increased epidermal and endodermal cell area, thicker cortical region more vessel numbers and enlarged meta-xylem and proto-xylem vessels. These structural changes adapted by populations in dry habitats (CSS and JAB) are prominent adaptive features to tolerate water deficiency.

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