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Full Length Article

Structural and Functional Modifications in Osmoregulation for Ecological Success in Purple Nutsedge (*Cyperus rotundus*)

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Abstract

Cyperus rotundus (family Cyperaceae) also known as purple nutsedge is a perennial weed with creeping scaly rhizomes that arise from tubers. Eight populations of *C. rotundus* from different ecozones in the Punjab were investigated to evaluate morphological, anatomical and physiological response to soil and environmental conditions. Anatomical characteristics are strongly responsive to environmental conditions. *C. rotundus* is well adapted to environmental heterogeneity, *i.e*., agricultural fields, dryland salinity, salt marshes, and desert and semi-desert climates. Its ecological success is because of plasticity in structural and functional features to adapt to different environmental conditions and water conservation via storage parenchyma, wide xylem vessels for conduction of solutes, sclerenchyma and stomatal features for minimizing water loss. Populations from drier habitats showed intensive sclerification in root vascular region, large metaxylem vessels, vascular area and stomatal density. Population from salt marsh had high accumulation of free proline, free amino acids, Ca^{2+} and aerenchyma formation in roots and stem, vascular bundle area and number in stem, thicker leaves and well-developed bulliform cells. *C. rotundus* populations showed high degree of plasticity in morpho-anatomical and physio-biochemical characteristics, which provide this species a great potential to acquire a variety of habitats. Each population showed specific modifications regarding morpho-anatomical and physiological characteristics, which indicated its adaptability potential to a specific environmental condition.

Keywords: Aerenchyma; Bulliform cells; Plasticity; Sclerification; Water conservation

Introduction

Purple nutsedge (*Cyperus rotundus* L.) is a perennial weed with creeping scaly rhizomes that arise from tubers. It is widely distributed all over the world, but native to Africa, temperate and tropical Asia and Europe (Uddin *et al.*, 2006). It is among the most abundant weeds in Pakistan and found in Indus plains during summer season in important field crops such as sugarcane, cotton and maize. It is highly competitive in nature and cause significant reduction in crop yield (Bryson *et al.*, 2003). Distributional range of this species is quite diverse, which is adapted to a variety of environmental conditions like deserts and semi-deserts, wetlands, salt-affected areas, forests, roadsides *etc*. (Uddin *et al.*, 2006).

Purple nutsedge grows in wide variety of environmental conditions of Pakistan such as polluted soil, sandy deserts, nutrient poor soil, saline lakes and most abundantly in aquatic habitats, so it has to develop many structural and functional modifications to cope with adverse soil conditions (Ratcliffe, 1995). Stem tubers of this species have greater reserves of soluble sugar and increased amylase activity that may play a role in its wide distributional range (Pena-Fronteras *et al.*, 2009). Anatomical modifications like root and stem aerenchyma facilitate diffusion of oxygen, enabling this species to colonize even under anaerobic conditions (Benz *et al.*, 2007).

Abiotic stress causes reduction in water availability to plants, *i.e*., plants exposed to drought or physiological drought (Senthil-Kumar *et al.*, 2003). For examples, salinity adversely affects cell wall thickening and cell enlargement (Minic and Jouanin, 2006), and reduction in storage parenchyma (Dolatabadian *et al.*, 2011). Sclerification in aerial parts and roots, epidermal and cuticle thickening (Hose *et al.*, 2001), thick leaves, excretory hairs, bulliform cells (Alvarez *et al.*, 2008) and leaf pubescence are some characters that indicate presence of environmental stresses, particularly drought (Naidoo *et al.*, 2012). Under waterlogged conditions of the soil, gas exchange ability increases by the high stomatal density, presence of aerenchyma and size of stomatal complex (Muhlenbock *et al.*, 2007).

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High salinity in the soil solution may negatively affect the growth of plants either through osmotic inhibition of water uptake by roots or specific ion effects (Munns, 2002). Many plants accumulate reasonable amount of free proline that play a crucial role to resist the side effects of salt stress (Cha-Um and Kirdmanee, 2011). Ion homeostasis is one of the physiological processes that help the plants to grow and flourish in saline wetlands (Zhu, 2003). Homeostasis includes toxic ions accumulation (Hameed *et al.*, 2009), selective uptake of required ions (Flowers and Colmer, 2008), excretion of the unwanted ions by leaf hairs, trichomes, leaf sheath and other excretory organs (Ramadan and Flowers, 2004; Naz *et al.*, 2009).

This study was conducted to identify structural features that regulate osmoregulation in purple nutsedge, which are important for this species to inhabitate a variety of habitat types. It was therefore hypothesized that plasticity in structural and functional features is responsible for ecological success in this species.

Materials and Methods

Eight populations of *C. rotundus* were collected from different ecological regions of the Punjab Province, *viz*., wheat fields (Faisalabad), cotton fields (Haroonabad), barren area (Gatwala), canal bank (Treemu Headworks), saline wasteland (Sahianwala), Thal Desert (Bhakkar), Cholistan Desert (Rahimyar Khan) and Kalar Kahar Lake (Chakwal) in the Punjab. Soil physicochemical characteristics are presented in Fig. 1.

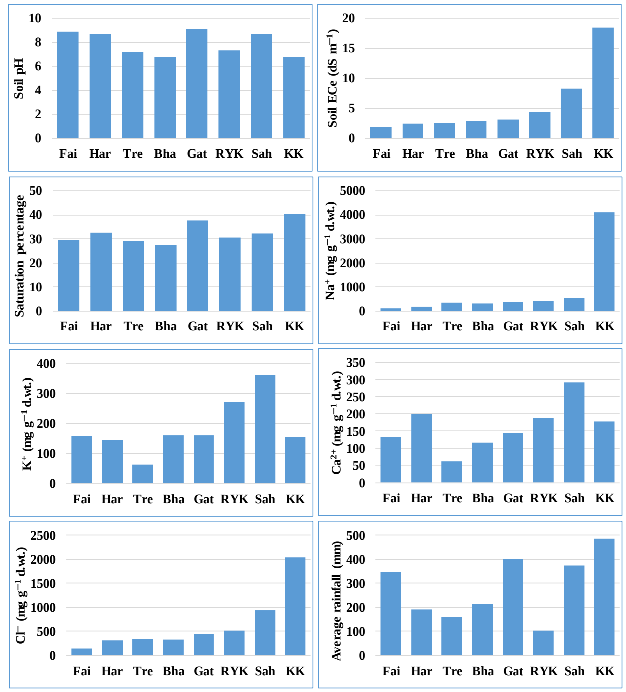

Fig. 1: Soil physicochemical characteristics of *Cyperus rotundus* collection sites from the Punjab

Organic Osmolytes

Fresh samples were collected in plastic zipper bags, kept in icebox and brought back to laboratory for total free amino acids, free proline content and total soluble proteins. Free proline was estimated by homogenizing fresh leaves with sulfo-salicylic acid (Bates *et al.*, 1973), filtered and then added ninhydrin solution. The solution was kept at 100°C for one hour and then absorbance was recorded at 520 nm. For determination of total free amino acids, ninhydrin was added to plant extract, covered it with in aluminium foils, heated in boiling water for twenty minutes and then cooled by pacing in cold water (Moor and Stein, 1948). It was then diluted, incubated again and optical density was recorded at 570 nm.

Total soluble proteins were recorded by taking 0.2 g of plant material in phosphate buffer (Lowry *et al.*, 1951). Copper reagent was prepared by 3 solutions, A, B and C. Solution A was prepared in distilled water by adding Na_2CO_3, NaOH and sodium potassium titrate. For solution B, copper sulphate was added to distilled water and for solution C, solution A and B was were mixed. Folin phenol reagent was prepared by adding sodium tungstate and sodium molybdate in distilled water. Orthophosphoric acid and HCl then added to the solution and then lithium sulfate and few drops of Br_2 was added, boiled it, cooled and diluted. Bovain serum albumin was also prepared in distilled water. All these solutions were mixed according to the procedure and optical density was recorded at 620 nm. Anthron reagent was added to plant material for total soluble sugars (Yemm and Willis, 1954). The solution was heated in boiling water for 10 minutes, then cooled in icy water and incubated at room temperature. Optical density was recorded at 625 nm on spectrophotometer (Hitatchi, 220, Japan).

Gas Exchange Parameters

Measurements of transpiration rate (*E*), net assimilation rate (*A*), sub-stomatal CO_2 concentration (C*i*), stomatal conductance (*gs*) and water use efficiency (A/E) were taken using LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddesdon, England). Readings were recorded during 9:00 a.m. to 11:00 a.m. Atm. pressure 99.9 k Pa; molar flow of air per unit leaf area 403.3 mmol m^{-2} s^{-1}; water vapor pressure of chamber 6.0 to 8.9 mbar, shoot temp. 28.4 to 32.4°C, PAR 1711 μmol m^{-2} s^{-1}, ambient CO_2 conc. 352 μmol mol^{-1}, ambient temp. 22.4 to 27.9°C.

Shoot Water Relations

Scholander-type pressure chamber was used from 8:00 am to 10:00 am for the measurement of shoot water potential of each species collected from six habitats. The shoot that

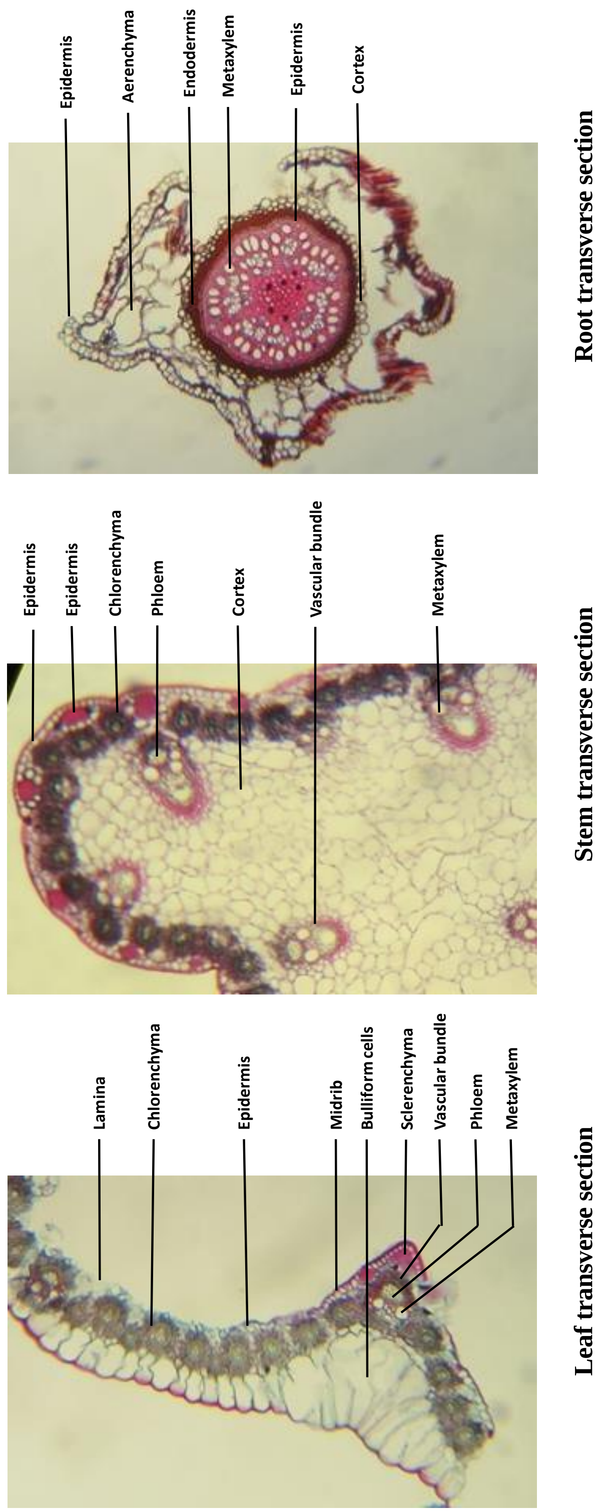

Fig. 2: Measurement details of different tissue systems in *Cyperis rotundus* populations

was used for water potential, then frozen in freezer for seven days at -20°C. The frozen plant material was melted and then extraction of cell sap performed with the help of disposable syringe. The extracted sap was then subjected to vapor pressure osmometer (Wescor 5500) to determine the shoot osmotic potential. Then turgor potential was calculated by subtracting values of osmotic potential (Ψ_s) from the water potential (Ψ_w).

Plant Ionic Content

Root and shoot ground dried material were digested with hydrogen per oxide and sulphuric acid for analyzing different nutrients (Ca^{2+}, Na^+ and K^+) according to the method used by Wolf (1982). Values of K^+, Na^+ and Ca^{2+} were recorded on flame photometer was then compared with standard curve and quantity of each ion computed.

Anatomical Parameters

For anatomical studies, root, stem and leaf samples were detached from the plants and immediately preserved in formalin acetic alcohol (FAA) solution following Ruzin (1999) for anatomical studies. Permanent slides were prepared by free hand sectioning technique. A series of ethanol grades were used for dehydration of transverse sections and biological stains safranin and fast green were used for developing contrast among different tissue systems. The sections were then photographed by camera-equipped compound microscope (Nikon 104, Japan). Measurements were taken by ocular micrometer. Details of measurements for anatomical characteristics are presented in Fig. 2.

Statistical Analysis

Samples for morpho-anatomical and physiological characteristics were taken from three different sites and the data were subjected to one-way analysis of variance using Microsoft Excel.

Results

Morphological Characteristics

Shoot fresh weight varied significantly in all populations of *C. rotundus.* Maximum shoot growth was recorded in plants that were growing as a weed in cultivated crops like wheat (Faisalabad) and cotton (Haroonabad). The minimum growth was recorded in the desert populations at Bhakkar (Thal Desert) and Rahimyar Khan (Cholistan Desert). Root fresh weight showed differential response as the growth was maximum in desert area of Thal and Cholistan (Table 1), while in populations from wetter habitats like canal bank and salt marshes showed significantly reduced root fresh weights.

Gas Exchange Parameters

Variation was found for gas exchange characteristics significantly among *C. rotundus* populations (Table 1). The highest net CO_2 assimilation rate, transpiration rate and sub-stomatal CO_2 concentration was observed in population collected from wheat field (Faisalabad). Maximum stomatal conductance was observed in Kalar Kahar population, which varied not-significantly from the canal bank population at Treemu. Highest water use efficiency was observed in Sahianwala population from saline wetland and Gatwala barran area. Populations from wheat field Faisalabad also showed high water use efficiency (5.0), whereas this character in all other populations ranged from 3.9 to 4.3, which was significantly lower than the Faisalabad population.

Water Relation Traits

The maximum shoot water potential was recorded in Kalar Kahar population, which varied not significantly from Sahianwala and Faisalabad population (Table 1). Not significant differences were recorded in other population regarding shoot water potential. Highest shoot osmotic potential was observed in Cholistan population that showed not significant differences from Kalar Kahar and Bhakkar populations. Highest turgor potential was observed in Cholistan population, followed by the Thal population. The minimum value for this characteristic was recorded in wheat and cotton fields populations, which were significantly lower than all other populations.

Ionic Content

Kalar Kahar population surpassed all other population regarding shoot and root Na^+. Shoot Na^+ varied from 26.2 to 32.7 mg g^{-1} d.wt., whereas root Na^+ from 15.1 to 18.0 mg g^{-1} d.wt. in other populations of *C. rotundus* (Table 1). Shoot Ca^{2+} varied significantly at $P < 0.05$, but not significant differences were recorded for root Ca^{2+} among *C. rotundus* populations. The maximum value for shoot Ca^{2+} was recorded in cotton field and salt marsh populations. Shoot K^+ was the maximum in Cholistan population, while its minimum was recorded in Thal population. This characteristic varied slightly in all other populations. Root K^+ was the maximum in Cholistan desert, which varied not significantly from wheat field, barren area, Thal Desert, saline wetland and salt marsh populations.

Organic Osmolytes Accumulation

Highest accumulation of total free amino acids, free proline and total soluble sugars were found for Kalar Kahar population, which were significantly higher than other populations (Table 1). Highest total soluble proteins were in saline wetland population and not varied significantly from cotton field.

Root Anatomical Characteristics

Significant variation was observed in root morphological

Table 1: Morphological and physiological characteristics of *Cyperus rotundus* collected from different ecological regions of Punjab

	Wheat field	Cotton field	Barren area	Canal bank	Thal desert	Cholistan desert	Saline wetland	Salt marsh	
	Fai	Har	Gat	Tre	Bha	RYK	Sah	KK	F-ratio
Morphological characteristics									
Shoot fresh weight (g plant^{-1})	31.17a	32.25a	28.17b	22.17c	15.53d	15.29d	23.04c	21.11c	604.53***
Root fresh weight (g plant^{-1})	22.13b	20.22bc	19.47c	15.19d	26.03a	24.97a	12.97de	12.22e	537.04***
Gas exchange parameters									
CO_2 assimilation rate (μmol m^{-2} s^{-1})	13.3a	8.6c	10.9b	8.3c	8.5c	9.1c	12.4a	8.3c	6.40**
Transpiration rate (mmol m^{-2} s^{-1})	2.6a	2.2a	1.9b	2.1a	1.9b	2.1a	2.1a	2.1a	6.09**
Stomatal conductance (mmol m^{-2} s^{-1})	104.7d	106.7d	148.7b	153.7ab	106.7d	115.3c	109.01d	157.3a	212.42***
Sub-stomatal CO_2 conc. (μmol mol^{-1})	296.7a	151.7e	203.3c	196.7.3c	135.01f	180.7d	262.7b	174.01e	81.82***
Water use efficiency	5.0b	3.9c	5.6a	4.1c	4.3c	4.2c	5.9a	3.9c	4.80**
Water potential									
Shoot water potential (-Mpa)	1.40bc	1.52ab	1.61a	1.61a	1.49ab	1.50ab	1.40bc	1.31c	6.26**
Shoot osmotic potential (-Mpa)	1.12b	1.23a	1.09b	1.22a	0.63de	0.53e	0.92c	0.73d	25.72***
Shoot turgor potential (Mpa)	0.29e	0.28e	0.51c	0.41d	0.87b	0.97a	0.53c	0.57c	20.26***
Ionic content									
Shoot Na^+ (mg g^{-1} d.wt.)	28.0c	29.0bc	32.7b	33.0b	26.2d	30.2b	30.3b	49.0a	9.7***
Root Na^+ (mg g^{-1} d.wt.)	18.0b	17.0bc	15.1d	15.5d	15.7d	16.2cd	17.3bc	63.6a	176.67***
Shoot Ca^{2+} (mg g^{-1} d.wt.)	23.2ab	25.0a	20.5c	22.0bc	19.8c	19.3c	20.3c	25.0a	3.13*
Root Ca^{2+} (mg g^{-1} d.wt.)	6.3bc	6.5bc	5.7c	6.0bc	7.5ab	6.6bc	6.5bc	8.7a	2.48^{NS}
Shoot K^+ (mg g^{-1} d.wt.)	8.5ab	8.0b	6.3bc	5.0cd	4.0d	10.0a	6.7bc	8.0b	6.15**
Root K^+ (mg g^{-1} d.wt.)	21.3a	18.0b	21.7a	16.3b	20.8a	22.3a	22.0a	20.8a	3.78*
Organic osmolytes									
Total free amino acids (μg g^{-1} f. wt.)	1140.0d	1491.8b	559.0g	1310.4c	793.3f	910.7e	509.1g	1638.0a	33.59***
Proline (μmol g^{-1} f. wt.)	120.9d	186.3b	151.9c	159.5c	141.5c	157.5c	116.07d	211.1a	314.59***
Total soluble proteins (μg g^{-1} f. wt.)	886.0c	1094.7a	1059.2b	835.1d	852.3d	846.7d	1100.8a	1052.7b	48.52***
Total soluble sugars (mg g^{-1} d. wt.)	23.3e	28.4b	25.7c	23.2e	22.7e	24.3d	28.7ab	29.7a	6.65***

Means sharing similar letters in each row are statistically not significant
* = Significant at $P < 0.05$, ** = significant at $P < 0.01$, *** = significant at $P < 0.001$, NS = not significant
Fai: Faisalabad, Har: Haroonabad, Gat: Gatwala, Tre: Treemu, Bha: Bhakkar, RYK: Rahimyar Khan, Sah: Sahianwala, KK: Kalar Kahar

characteristics of *C. rotundus* at selected sites (Table 2 and Fig. 3). The maximum root and vascular bundle thickness were recorded in Cholistan Desert population followed by Thal desert population. Cholistan population depicted the narrowest vessels among all other populations. The maximum epidermal thickness was recorded in canal bank population, for cortical thickness in wheat field population, and for cortical cell area in Thal desert population. Barran area population showed the maximum value for aerenchymatous area and saline wetland population for metaxylem area. Wheat field population had the minimum epidermal thickness and cortical cell area, while canal bank population depicted the minimum vascular bundle thickness and aerenchymatous area. Thal desert population showed the minimum value cortical thickness and saltmarsh population for root thickness.

Stem Anatomical Characteristics

Maximum stem thickness and epidermal thickness was observed for Haroonabad population exceeding all other habitats (Table 2 and Fig. 3). This population showed the minimum values for metaxylem area and sclerenchymatous thickness. Cholistan desert population possessed the maximum cortical cell area and metaxylem area. The maximum value for sclerenchymatous thickness was observed in canal bank population, for vascular bundle area in barren area and for aerenchymatous area in saltmarsh population. Thal desert population exhibited the second best of epidermal thickness, metaxylem area, sclerenchymatous thickness and aerenchymatous area.

Leaf Anatomical Characteristics

Thal Desert population showed the maximum value for lamina thickness, cortical cell area, vascular bundle area and metaxylem area (Table 2 and Fig. 3). Epidermal thickness and stomatal density were also high in this population. Cholistan Desert population possessed the maximum midrib thickness, bulliform thickness and stomatal density. This population also had large vascular bundles and wide metaxylem vessels. Barren area population had maximum stomatal area and saline wetland population for epidermal thickness. Minimum stomatal area was observed in Cholistan Desert population and stomatal density in cotton field population.

Specific Anatomical Modifications

The most variable characteristic of root was number of metaxylem vessel, which greatly increased in desert populations from Bhakkar and Cholistan (Table 3). Populations from saltmarsh and wheat field showed only two large vessels in vascular tissue, while these ranged from 3-4 in all other populations. Another prominent feature of Cholistan population was storage parenchyma in

Table 2: Anatomical characteristics of *Cyperus rotundus* collected from different ecological regions of Punjab

	Wheat field	Cotton field	Barren area	Canal bank	Thal Desert	Cholistan desert	Saline wetland	Saltmarsh	
	Fai	Har	Gat	Tre	Bha	RYK	Sah	KK	F-ratio
Root Anatomy									
Root thickness (μm)	269.6de	326.8c	294.1cb	245.1ef	392.2b	449.4a	290.0cb	212.4f	272.66***
Epidermal thickness (μm)	18.7f	79.0cd	57.0e	126.2a	107.3b	105.2b	82.2c	69.6de	19.47***
Cortical thickness (μm)	106.2a	81.7bc	65.4de	73.5cd	57.2h	61.3e	102.1a	85.8b	14.98***
Cortical cell area (μm^2)	57.0h	189.2	367.5	220.6	470.3	315.0	142.0	107.3	111.86***
Metaxylem area (μm^2)	209.6a	157.7b	113.6cd	127.3c	105.2de	52.8f	210.1a	94.8e	31.72***
Vascular bundle thickness (μm)	40.9de	39.2e	65.4c	31.0e	81.7b	138.9a	49.0d	34.3e	5868.85***
Aerenchyma Area (μm^2)	419.9g	787.0f	2727.5a	393.7g	1180.4d	1049.3e	1678.6c	2124.4b	586.19***
Stem Anatomy									
Stem thickness (μm)	857.9b	964.1a	825.2e	531.1f	563.7e	939.6a	694.5d	669.9d	1225.71***
Epidermal thickness (μm)	5.7d	8.2a	4.1de	6.5c	7.4b	4.9de	7.4b	6.5c	8.35**
Cortical cell area (μm^2)	315.0f	944.4b	440.9d	393.7e	382.2e	1180.4a	503.8c	472.4cd	232.56***
Vascular bundle area (μm^2)	472.4d	283.6f	897.2a	671.7b	551.0c	380.6e	235.3g	688.4b	133.37***
Metaxylem area (μm^2)	126.2e	110.5f	157.7d	118.4ef	223.2b	336.0a	195.6c	173.4d	39.19***
Sclerenchyma thickness (μm)	24.5b	10.6d	16.3c	32.7a	31.0a	30.2a	26.1b	15.5c	305.09***
Aerenchyma area (μm^2)	4181.7b	1678.6d	2753.7c	4015.6b	20488.7a	0.0e	1794.0d	39054.5a	329.00***
Leaf Anatomy									
Leaf midrib thickness (μm)	98.0f	114.4e	126.6d	130.7d	147.1c	200.2a	122.6d	179.7b	52.87***
Leaf lamina thickness (μm)	57.2c	53.1c	28.6d	57.2c	122.6a	77.6b	55.6c	80.1b	3440.52***
Bulliform thickness (μm)	32.7e	44.9d	53.1c	42.5d	57.2b	98.0a	39.2d	73.5b	2060.57***
Epidermal thickness (μm)	8.2b	4.9e	3.3f	6.5d	8.2b	7.4e	9.8a	6.5d	18.80***
Cortical cell area (μm^2)	115.7b	63.3f	102.6c	86.9e	294.0a	88.5de	47.6g	94.8d	73.11***
Vascular bundle area (μm^2)	1101.7g	839.5f	881.4f	1336.7d	3671.5a	3440.8b	1238.1e	1888.4c	989.21***
Metaxylem area (μm^2)	26.6g	57.0e	79.0d	37.1f	157.7a	126.2b	52.8e	86.9c	36.41***
Stomatal area (μm^2)	524.8d	577.3c	1227.6a	330.8e	323.4e	275.7f	734.6b	755.6b	229.90***
Stomatal density	18.0e	16.0e	21.0d	25.0c	39.0a	40.0a	24.0c	30.0b	245.09***

Means sharing similar letters in each row are statistically not significant

* = Significant at $P < 0.05$, ** = significant at $P < 0.01$, *** = significant at $P < 0.001$, ns = not significant

Fai: Faisalabad, Har: Haroonabad, Gat: Gatwala, Tre: Treemu, Bha: Bhakkar, RYK: Rahimyar Khan, Sah: Sahianwala, KK: Kalar Kahar

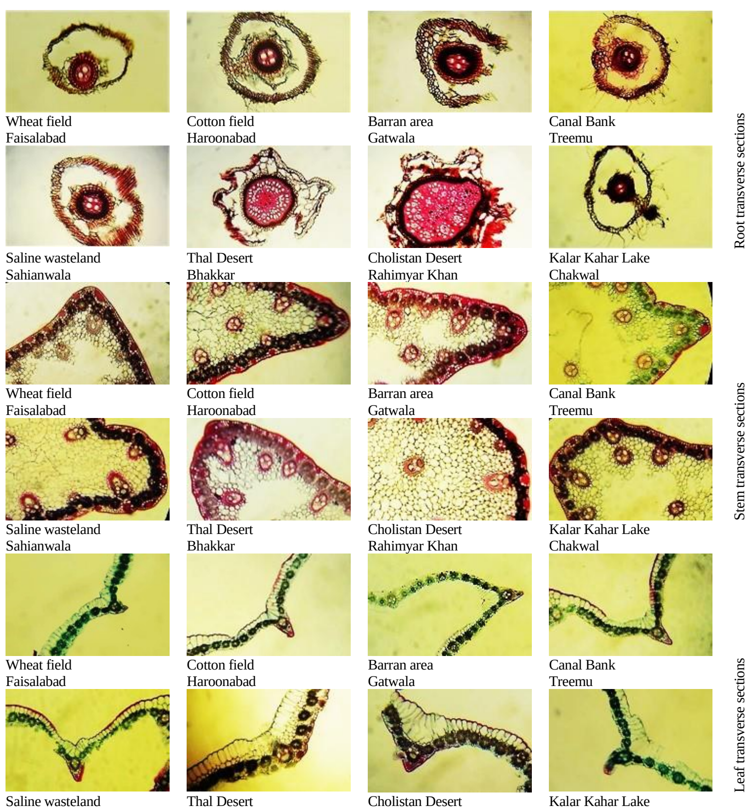

Fig. 3: Root, stem and leaf anatomical characteristics of *Cyperus rotundus* populations collected from different ecozones in the Punjab

Table 3: Response of morpho-physiological and anatomical characteristics in *Cyperus rotundus* populations from diverse regions in the Punjab

Habitats	Morphology	Physiology		Root anatomy	Stem anatomy	Leaf anatomy
Wheat field Faisalabad	Shoot biomass	*A* C*i* S-K^+	*E* R-Na^+	Cortical thickness Metaxylem		Cortical cell area
Cotton field Haroonabad	Shoot biomass	*E* S-Ca^{2+} Proline	ψ_s AA Protein		Stem thickness Epidermis Cortical cell area	
Barran area Gatwala		WUE	ψ_w	Cortical cell area Aerenchyma	Vascular bundle	Stomatal area
Canal bank Treemu		G*s* ψ_s	ψ_w S-Na^+	Epidermis	Sclerenchyma	
Thal Desert Bhakkar	Root biomass	ψ_p	R-Ca^{2+}	Root thickness Epidermis Cortical cell area Vascular bundle	Epidermis Metaxylem Sclerenchyma Aerenchyma	Lamina thickness Epidermis Cortical cell area Vascular bundle Metaxylem Stomatal density
Cholistan Desert Rahimyar Khan	Root biomass	ψ_p R-K^+	S-K^+	Root thickness Vascular bundle	Stem thickness Cortical cell area Metaxylem	Midrib thickness Bulliform Vascular bundle Metaxylem Stomatal density
Saline wetland Sahianwala		*A* WUE Protein	C*i* S-K^+ Sugars	Cortical thickness Metaxylem		Epidermis
Saltmarsh Kalar Kahar		G*s* R-Na^+ R-Ca^{2+} Free proline	S-Na^+ S-Ca^{2+} AA Sugars	Aerenchyma	Vascular bundle Aerenchyma	Midrib thickness Lamina thickness Bulliform Stomatal area

A:, net CO_2 assimilation rate, AA: total free amino acids, C*i*: sub-stomatal CO_2 concentration, *E*: transpiration rate, G*s*: stomatal conductance, Proline: proline content; Protein: total soluble proteins, R-Ca^{2+}, Root calcium, R-K^+: root potassium, R-Na^+: root sodium, S-Ca^{2+}: shoot calcium, S-K^+: shoot potassium, Sugar: total soluble sugars, WUE: water use efficiency, ψ_p: turgor potential, ψ_s: osmotic potential, ψ_w: water potential

stem, which was greatly increased and composed of large loosely packed cortical cells. Chlorenchyma was compressed and large sclerenchymatous bundles scattered all over the chlorenchyma. Desert population (Cholistan and Thal) showed significantly enlarged bulliform cell on adaxial leaf surface, especially those recorded in Cholistan population on midrib region. Shape of midrib also varies greatly in *C. rotundus* populations, which ranged from sharp pointed to triangular in Cholistan Desert population and rounded in Thal Desert population.

Discussion

Anatomical characteristics are generally susceptible to prevailing environmental conditions and therefore, strongly responsive to biotic and abiotic stresses (Naskar and Palit, 2015). Water conservation is the main strategy under drought or physiological drought caused by other environmental stresses, for which plants must modify anatomically along with morphological and physiological modifications (Alvarez *et al.*, 2008; Sun *et al.*, 2018). Under limited water availability, water conservation is either by water storage in parechymatous tissues, or efficient conduction through broader xylem vessels, and prevention of water loss by mechanical tissue (Micco and Aronne, 2012).

Annual rainfall was moderately good at Faisalabad and sufficient for growth enhancement of purple nutsedge. ECe of this site was the minimum and maximum fresh weight among all populations. High net assimilation rate and sub-stomatal CO_2 concentration justifies shoot fresh weight in this population, as also earlier been reported by several authors (Stoeva and Kaymakanova, 2008; Qados, 2011; Ashraf and Harris, 2013). Simultaneously, transpiration rate was also high which lowers water use efficiency (Omamt *et al.*, 2006), but this may not affect its growth and development as there is sufficient water availability. Water conduction is not a prime strategy of Faisalabad population because it is not exposed to drought or physiological drought conditions (Corrêa *et al.*, 2017). It showed medium to low development of parenchyma in root, stem and leaves, which again justified non-stressful conditions of the habitat (Elhalim *et al.*, 2016). Moreover, tissues that involve in prevention of water loss through plant body, such as sclerification near epidermal region and bulliform cells were not much developed (Micco and Aronne, 2012). Although stomatal density was very much reduced, this may be an advantageous for this population (Camargo and Marenco, 2011). Cortical cells of leaves, however, were large-sized to maintain the turgor under higher transpiration rate (Bell and O'Leary, 2003).

Haroonabad is situated near Cholistan desert so it receives low rainfall annually, but its ecotypes managed to have better shoot fresh weight as its soil is good in saturation percentage. High turgor potential in this population may cause better growth (Benzarti *et al.*, 2014) and this may ensure high shoot fresh weight than other populations (González *et al.*, 2012). Better growth has also been related to Ca^{2+} concentration, *e.g.*, Cabot *et al.* (2009) and Jiang *et al.* (2013) and Haroonabad population was rich in shoot Ca^{2+}. Under semi-arid conditions near Cholistan Desert, free amino acids, proteins and soluble sugars produced in excess to combat with ROS under abiotic stress (Gupta and Huang, 2014). Stem epidermis is very thick, and this may prevent water loss, which is an important strategy under water deficit conditions. Enlarged stem diameter, which is mainly due to storage parenchyma, help the plant to store more water. Metaxylem vessels are the main conducting tissue (Corrêa *et al.*, 2016) and their size is positively related to water and nutrients conduction efficiency (Smith *et al.*, 2013). Narrow xylem vessels in this population are extremely beneficial to this population as vessels are less prone to collapse under limited water supply (Zhaosen *et al.*, 2014). Low stomatal density indicates plant adaptation to arid and semi-arid lands (Xu and Zhou, 2008), which can regulate transpiration rate and hence, extremely useful to ensure water conservation (Camargo and Marenco, 2011).

Gatwala is a forest plantation located near Faisalabad with best annual rainfall (402 mm). Plants of this habitat were tall, and require sufficient moisture for their growth and development. Photosynthetic rate was reasonably high and as was the case with water use efficiency. All these contribute towards increased biomass in Gatwala population (Ventura *et al.*, 2014). Large cortical cells in roots are capable of storing more water (Bell and O'Leary, 2003), and hence can withstand drought periods more successfully (Senthil-Kumar *et al.*, 2003). Aerenchyma formation in the roots may provide additional advantage to this ecotype, as root aerenchyma may withstand hypoxic soil conditions. Aerenchyma formation has been reported in plants facing physiological droughts (Shimamura *et al.*, 2010; Grigore *et al.*, 2014). Vascular bundles contain broader vessels, this may involve in efficient water conduction when moisture availability is sufficient (Smith *et al.*, 2013). Stomatal size was the maximum, and larger stomata can enhance photosynthetic efficiency of a plant (Bray and Reid, 2002), as well as transpiration rate (Akram *et al.*, 2002).

Treemu receives low rainfall annually and population collected from canal bank and faces no water shortage at all. Among physiological parameters, low values for CO_2 assimilation rate and transpiration rate, but stomatal conductance was high. Root epidermis is thick and stem periphery is heavily sclerefied. Sclerification is often related to water conservation (Abernethy *et al.*, 1998), in addition to providing mechanical strength to plant tissues (Al-maskri *et al.*, 2013; Nawaz *et al.*, 2013).

The Bhakkar population was collected from Thal Desert, where average rainfall is very low. Plant were poorly developed, but root fresh weight was the maximum, as in the present study. Root growth is generally better in plants growing in desert condition like Thal Desert (Senthil-Kumar *et al.*, 2003). This population showed the maximum of many root, stem and leaf anatomical characteristics. For example, proportion of mechanical, dermal, vascular and storage tissues. All these are vital for water conservation, by either checking water loss from plant body (Abernethy *et al.*, 1998) or storing water inside plant body (Bell and O'Leary, 2003). Plants colonizing desert climates requires such structural modifications for their survival (Farooq *et al.*, 2009).

Rahimyar Khan population was collected from Cholistan Desert with minimum annual rainfall. Root fresh weight was the maximum but shoot development was poor, as for Bhakkar population. This population also showed anatomical modification like increased vascular tissues and cortical parenchyma, which increased root and stem thicknesses. Moreover, bulliform cell were well developed, and this is extremely important for minimizing transpiration rate when water is a vital commodity (Hameed *et al.*, 2012; Ahmad *et al.*, 2015). High proportion of storage parenchyma (succulence) is again critical for desert plants, which may guarantee survival in extremely harsh arid climates (Farooq *et al.*, 2009).

Saline soils of Sahianwala restricted growth of root in *C. rotundus* population (Hameed*et al.*, 2013; Younis *et al.*, 2014). High photosynthetic activity and low transpiration rate contributes towards enhanced water use efficiency (Omamt *et al.*, 2006). No prominent change in anatomical characteristics observed in this population, but leaf epidermal thickness was the maximum. Epidermis is a protective layer and prevents internal tissue system from direct exposure to environmental hazards. Thicker and well-developed epidermis can solely protect a plant from desiccation, and therefore, the Sahianwala population survived successfully in physiological drought environments caused by high salinity (Akram *et al.*, 2002). High accumulation of total soluble proteins additionally benefitted this population that act as an osmoprotectant under limited moisture availability (Gupta and Huang, 2014).

Hypersaline Kalar Kahar lake received the maximum annual rainfall and population growing along the lake was the highest in degree of salinity tolerance. High accumulation of Na^+ is very much expected, but impact of this toxic ion may be neutralized by high uptake of Ca^{2+}. Similar finding has also been reported earlier by Cabot *et al.* (2009) and Jiang *et al.* (2013) and Hameed *et al.* (2013). This population showed high accumulation of osmoprotectants like free proline, free amino acids and soluble sugars and this again indicates high degree of salt tolerance of Kalar Kahar population (Gupta and Huang, 2014). Anatomically, aerenchyma formation in roots and stem, leaf midrib and lamina thicknesses, well developed bulliform cells, large but fewer stomata are vital for colonizing high salinities (Shimamura *et al.*, 2010; Grigore *et al.*, 2014).

Conclusion

C. rotundus populations showed high degree of plasticity in morpho-anatomical and physio-biochemical characteristics, which provide this species a great potential to acquire a variety of habitats. Populations of this species had been collected from diverse ecozones like deserts and semi-desert, salt marshes, fresh waters, dryland salinities and crop fields, *i.e*., moister to driest habitats. Each population showed specific modifications regarding morpho-anatomical and physiological characteristics, which indicated its adaptability potential to a specific environmental condition. Tolerant genes from this species can be isolated and incorporated in sensitive plant species in future research endeavors.

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