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Original Article

Dramatic changes in anatomical traits of a C_4 grass *Chrysopogon serrulatus* Trin. (Poaceae) over a 1000 m elevational gradient

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Abstract: *Chrysopogon serrulatus* (false beard-grass) is a dominant component of vegetation in the foothills of the Himalayas. To study whole plant morphology, individuals of *C. serrulatus* were collected from three plots at each of six locations spanning from 400 to 1,400 m. The population colonizing the highest elevation showed noticeable morphological modifications in different plant organs. Roots showed increased xeromorphy, specifically increased metaxylem number and area. In the stem, especially outside of the vascular tissue, there was intensive sclerification indicative of increased xeromorphy as a survival strategy. At the highest elevation, leaves were wider; aerenchyma formation and increased

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sclerification were noted in the leaf sheath; and a greater proportion of storage parenchyma was observed in the leaf blade, all indicators of succulence. In contrast, leaves at lower elevations had xeric morphological features such as increased epidermal thickness, sclerification and more developed metaxylem area. In conclusion, shifting of morphological features in below- and above-ground plant parts of *C. serrulatus* were linked to shifts in environmental factors along this elevation gradient, thus enabling the successful distribution of this species along this elevation gradient.

Keywords: Adaptive component; Storage parenchyma; Sclerification; Metaxylem; Aerenchyma

1 Introduction

The Potohar region is a part of Himalaya foothills located in northern side of the Punjab, Pakistan. It includes six districts Attock, Chakwal, Khushab, Mianwali, Jhelum and Rawalpindi. It includes semiarid and arid regions, mountain peaks, cooler and deep valley. This region features steep slopes, large elevational gradients, and associated dramatic changes in environmental conditions such as increased wind velocity, increased rainfall, decreased temperature, and changes in water and nutrient availability. These spatial differences support a wide range of biodiversity (Ahmad et al. 2010).

High elevations are generally accompanied by environmental stresses such as freezing temperature, low barometric pressure, high rain and snowfall, and strong winds (Jiang et al. 2021). Environmental changes can be divided into two categories, elevation specific (temperature, air pressure and solar irradiance) and elevation non-specific (moisture availability, wind velocity and growth period duration (Gupta et al. 2019). Climate can change rapidly within short distances with elevation, hence imposing high selection pressure on colonizing species (Bresson et al. 2011). In response, the inhabiting species modifies themselves morpho-anatomically and physio-biochemically for survival (Read et al. 2014). This may determine the wider distributional range of some species via genetically fixed attributes or through phenotypic plasticity (Byars et al. 2007).

Though most of the anatomical features are genetically fixed within a given species, they show significant variation with changing environments, particularly along elevational gradient (Weemstra et al. 2021). How and whether a species can acclimate or modify its morphology and physiology to meet environmental change will depend upon specific environmental factor or suite of environmental to factors that change with elevation. Xeromorphic characteristics such as reduced leaf size, more sclerification in soft tissues, increased hairiness, and decreased in density and size of stomata at higher elevations are the immediate response to water scarcity (De Carcer et al. 2017). The most important modifications for survival are increased lignin deposition (Mota et al. 2018), dense pubescence, increased mechanical tissue with large aerenchymatous cavities (Liu et al. 2010), and greater frequency of stomata on abaxial leaf surface (Mansoor et al. 2019). Other adaptive strategies in high elevation plants are differential xylem vessel formation (Motomura et al. 2007), high photosynthetic activity (Zhao et al. 2019) and thick leaves (Akinlabi et al. 2014).

Plants of the same species colonizing a wide range of elevations might show switching of adaptive strategies, i.e., mesomorphy, xeromorphy to succulence in accordance with the changing soil and climatic variables along elevational gradients. For example, the following changes have been noted in various species with increasing drought (Olsen et al. 2013, *Andropogon gerardii;* Honaine et al. 2016, *Cortaderia selloana;* Carmo-Silva et al. 2009 in mesic grasses; Keshavarzi 2020, Poaceae halophytes; Ho et al. 2019, *Spinifex littoreus*).

Chrysopogon serrulatus Trin. is a tufted perennial grass about 1 m tall. It is a dominant type of vegetation in mountainous region of south and east Africa, South Africa, Madagascar, temperate Asia, Indian subcontinent, indo-Chine and Malaysia (Magome et al. 2008; Ahmad et al. 2009; De Sanctis et al. 2013; Rawat et al. 2018; Andriarimalala et al. 2021). Elevation ranges from 400 to 3000 m at Pak-Afghan border (Nawaz et al. 2012; Haq and Badshah 2021). Ali (2016) categorized it herbaceous species, leaf form nanophyll (a leaf that is less than 25 mm long, or 25–225 mm^2 in area), lamina simple and it likes dry slopes, while its conservation status is "least concern" according to IUCN red list category (Ahmad et al. 2012). Pakistan and India, South Africa and Arabian Peninsula Economically, *C. serrulatus* is an important species. It is highly palatable and grazed by wildlife species round the year (Fourie et al. 2007;

Azeem et al. 2020). In dry seasons, this species is used to feed domestic livestock along with few other grasses (Andriarimalala et al. 2021). Ethnobotanically, it is referred for treatment of mouth and gastrointestinal disease (Radha et al. 2022).

This species dominates the entire Potohar Region, especially the Salt Range (Hameed et al. 2012; Nawaz et al. 2012; Fatima et al. 2022), and the western Himalayas in Azad Jammu and Kashmir (Furqan et al. 2019). In many countries of Asia, it frequently dominates due to its high tolerance to different environmental stresses (De Sanctis et al. 2013). Another prominent feature of this species is the presence of allelochemicals such as dibutyl phthalate, diphenylamine, and 4,4′-dioctyl and simiarenol (Chuah et al. 2014) that suppress growth of other colonizing plants. Despite its broad distributional range, this species is largely unexamined, specifically in Pakistan.

No literature is available on the adaptive features regarding this species, particularly in relation to elevational gradient. It was hypothesized that dominance of this species throughout the Potohar region might be due to high degree of plasticity in structural and functional features. Therefore, the study was conducted (i) to document its variations in ecological, morphological, physiological and anatomical traits with elevation, (ii) to elucidate those morphological features that respond to changes in elevation and environment and, thus, might facilitate the understanding of the widespread distribution of *C. serrulatus,* and (iii) to conclude whether different populations of *C. serrulatus* collected from varying altitudes switch between mesomorphy, xeromorphy and succulence to deal with environmental adversities imposed by altitudinal gradients.

2 Materials and Methods

2.1 Collection sites

Populations of *Chrysopogon serrulatus* were collected from six different elevations, 400, 600, 800, 1000, 1200 and 1400 m a.s.l., thus creating a 1000 m elevational gradient. Three different study sites were selected at each elevation, resulting in 18 total study sites. For a given elevation, the three study sites were separated by at least 20 km (Fig. 1).

2.2 Geographical data

Geographic data, i.e., coordinates, elevation, aspect and slope were collected for each study site. Coordinate and elevation data were recorded by GPS (Garmin eTrex Venture HC, USA). Aspect was measured by a magnetic compass (Pocket Geological Plastic Compass, USA) and slope by digital inclinometer (LD360 Electronic inclinometer, USA).

2.3 Environmental data

Climate data such as minimum and maximum annual temperatures, and annual rainfall were obtained from the Pakistan Meteorology Department (PMD) observatories positioned in different adjoining cities of the Potohar region (Fig. 1).

2.4 Soil physiochemical traits

Three study sites were selected at each of the six elevations. Five quadrats were laid at each of the three study sites and soil data were collected and then presented as an average for that elevation. Three soil samples from all five quadrats as shown in Fig. 1, were collected and mixed thoroughly and considered as one replication, i.e., 5 replications from each elevation. Soil was sampled at a depth between 15 and 20 cm for later analysis (see Fig. 1 for the within study location sampling design). The 200 g sample was oven-dried at 105°C for 6 days; distilled water was added to prepare a saturated paste for determination of ECe and ion concentration. Saturation percentage was measured by the following formula:

$$\text{Saturation percentage} = \text{Weight of the saturated paste} - \text{Dry weight of soil}$$

ECe of soil extract was measured by portable pH/Electrical Conductivity Meter (Model: WTW series InoLab pH/Cond 720) following methods described in Handbook No. 60 (USDA Laboratory Staff, 1954). Soil K^+ and Ca^{2+} were measured with flame photometer (Jenway, PFP-7) by running a series of standards (10-100 mg L^{-1}) for a comparison using a standard curve.

2.5 Ecological data

Ecological studies were conducted at each of the 6 different elevations to estimate distributional pattern and community structure. A single 100 m long

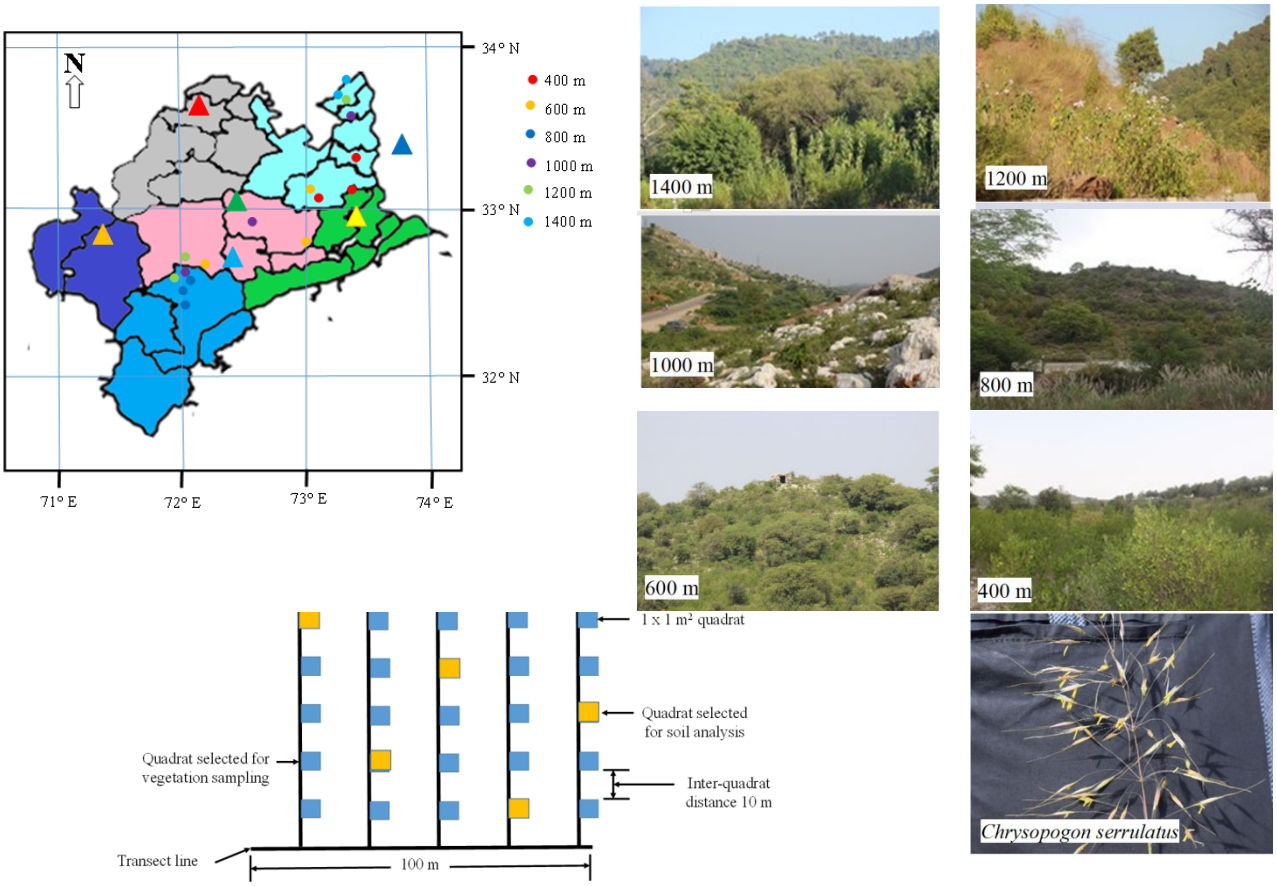


Fig. 1 Map of the Potohar region showing *Chrysopogon serrulatus* collection sites, Pakistan Meteorological Department Observatories and layout plan of quadrats for vegetation studies. Pakistan Meteorological Department Observatories: ▲Attock (code 41567), ▲Islamabad (41577), ▲Mianwali 41592), ▲Jhelum (41598), ▲ Jouharabad (41635), ▲Chakwal (41634).

transect line was laid out at each study site. Five 55 m sub-transects were laid on the main transect line, and 5 quadrats were along each sub-transect line, each separated by 10 m (total 25 quadrats were laid on main transect line) (Fig. 1). Density, frequency, cover, and importance value were measured following Greig-Smith (1983). Photographs of collection sites are presented in Fig. 1. Importance value was calculated by the formula:

$$\text{Importance value} = \text{Relative density} + \text{Relative frequency} + \text{Relative cover}$$

2.6 Morphological characteristics

At study sites, 5 transect lines were laid (Fig. 1). At each transect line, 5 quadrats were marked. One out of those five quadrats as indicated by orange colour was used to dug up three plants for morphological parameters. A total of 15 plants were collected from each study site. Shoot fresh weight was taken directly in the field using portable electric balance. Total plant height was measured from the base of stem to the top. Total leaves were counted from each plant. For leaf area, 5 fully mature leaves from the longest tiller of each plant (3 plants from each quadrat) were measured and area was calculated for each leaf. Later, the average leaf area was multiplied by total number of leaves to get total leaf area per plant. Plants were then dried in an oven for a week at 60°C and the short dry weight was measured.

2.7 Physiological parameters

Five quadrats at each elevation were laid in which soil samples were collected, averaged and used for final analysis. Three plants were randomly collected from each quadrat and averaged for analysis. The dried and powdered plant material (0.1 g) was

digested uquadrat andnc. sulphuric acid:H_2O_2 combination as described by Wolf (1982). Soil ECe, Na^+ and Cl^- were measured to assess salinity level of habitats. Macronutrients that involved in growth and development of plants, e.g., shoot K^+, Na^+ and Ca^{2+} were measured on a flame photometer (Jenway, PFP-7, United Kingdom). Soil Cl^- was estimated by chloride meter (Jenway, PCLM 3, Japan). Leaves collected from the field were kept in zipper bags, properly sealed and kept in a portable plastic ice-container. These leaves were used for the measurement of photosynthetic pigments. The method as described by Arnon (1949) was followed for determining chlorophyll *a* and *b* while that of carotenoids were determined by the methods of Davis (1976).

2.8 Anatomical parameters

Three plants were collected from each quadrat (5 quadrats from each elevation), sectioned for anatomical studies and averages were used for later analysis. The material (Fig. 2) was immediately preserved in leak-proof plastic bottles containing formalin acetic alcohol (FAA) solution. The material was then transferred to acetic alcohol solution after 48 h for storage. Free-hand sectioning technique was used for the preparation of permanent slides following Ruzin (1999). Plant sections were dehydrated using a series of ethanol grades and stained with safranin (lignified tissues) and fast green (parenchymatous tissues). The transverse sections were mounted on a slide using Canada balsam. Digital photographs were taken by a camera-equipped digital compound microscope. Micro-morphological data of the sections were taken by ocular micrometer pre-calibrated with a stage micrometer.

2.9 Statistical analysis

The data were subjected to analysis of variance (ANOVA) in completely randomized design with five replications, keeping the lowest elevation as a control following Steel et al. (1962). A principal component analysis (PCA) was performed using Canoco for Windows (v 4.5). Pearson's correlation coefficients were calculated, and path correlation analysis were constructed using a customized R code in R studios (Version 1.1.463 – © 2009-2018 RStudio, Inc.).

3 Results

3.1 Environmental data of collection sites

The mountains of the Potohar region have south- to east-facing slopes. The maximum temperature was nearly 40°C, except for the highest elevation, which had a maximum temperature of 25.8°C (Fig. 3). Hill slope was greatest at 1400 m and gradually decreased with decreasing elevation. The minimum temperature ranged between 2.7°C and 4°C up to 1200 m, while at 1400 m minimum temperature was −0.3°C. Annual rainfall increased exponentially with elevation, being less at low to moderate elevation (370 to 478 mm) and the maximum at 1400 m (1441 m). Soil ECe and ionic content (Na^+, K^+, Ca^{2+} and Cl^-) generally decreased with elevation.

3.2 Ecological distribution

All ecological parameters, relative density, relative frequency, relative cover and importance value decreased significantly at the highest elevation (1400 m). Relative density was the maximum at 1000 m, whereas relative frequency and importance value were the maximum at the lowest elevation (400 m). Relative cover was the maximum at two elevations, 400 and 1000 m (Table 1).

3.3 Morphology

Plant height, leaf area, and dry weight were the greatest at the three lowest elevations and least at the highest (Table 1). Other morphological characteristics such as leaves per plant, total leaf area, shoot fresh and dry weight was significantly higher at lower elevations.

3.4 Physiology

Plants growing at lower elevations showed maximum concentration of chlorophyll *a* and *b*, 2.4 and 0.89 mg g^{-1} f. wt., respectively (Table 1), which significantly reduced at the highest (1400 m) elevation (0.7 and 0.44 mg g^{-1} f. wt. respectively). Carotenoids concentration was the maximum (0.063 mg g^{-1} f. wt.) at 600 m. Shoot K^+ (18.2 mg g^{-1} d. wt.) and Ca^{2+} (28.7 mg g^{-1} d. wt.) were the highest at 800 m.

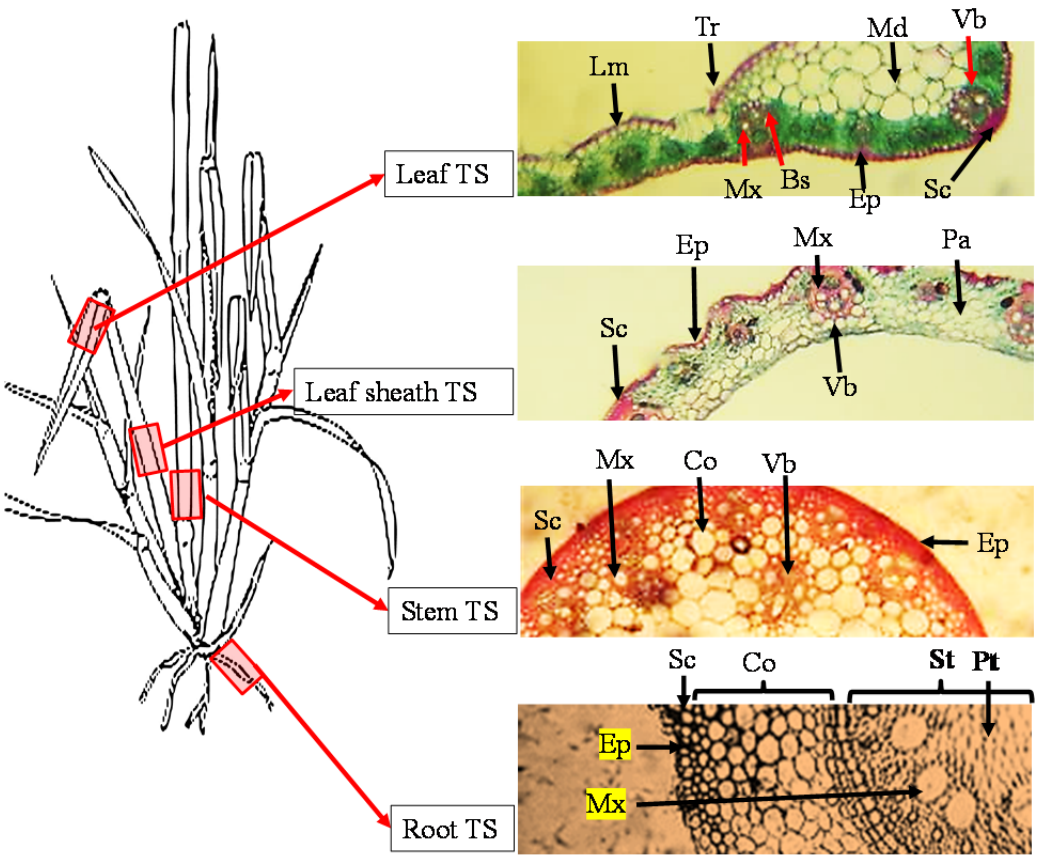


Fig. 2 Measurement details of anatomical studies in *Chrysopogon serrulatus* collected along elevation gradient. For morphological traits, 5 plants (replications) were randomly selected from each quadrat used for the vegetation studies at each elevation. The same plant was used for the estimation of ionic content and photosynthetic pigments.

Abbreviations: Bs–Bundle sheath cells, Co–Cortex, Ep–Epidermis, Lm–Lamina, Md–Midrib, Mx–Metaxylem, Pa–Parenchyma, Pt–Pith, Sc–Sclerenchyma, St–Stele, Tr–Trichome, Vb–Vascular bundle.

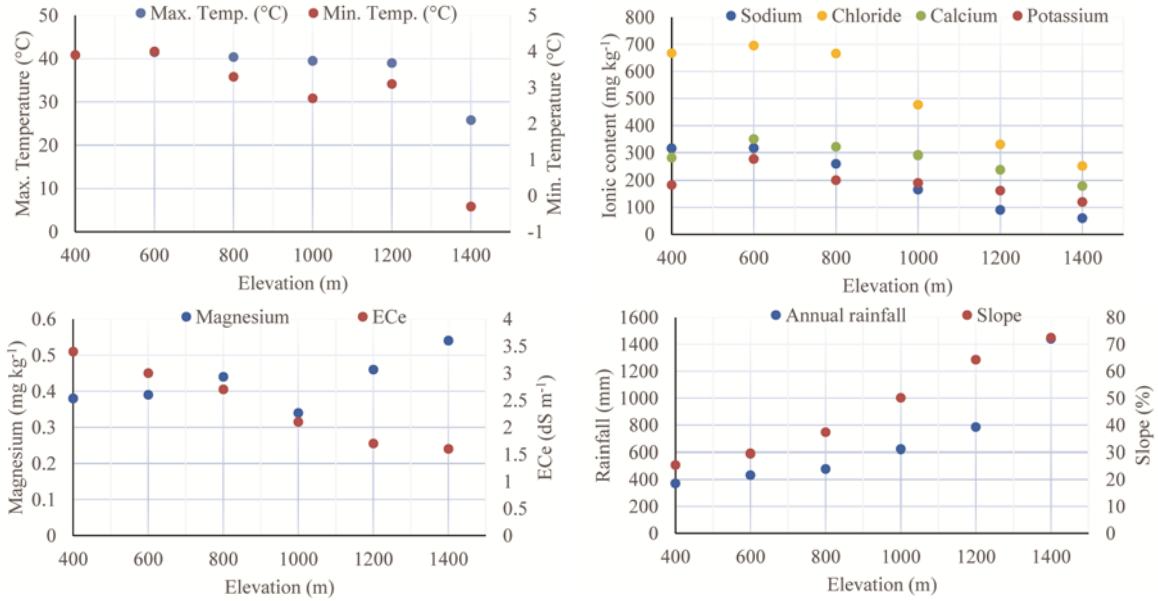


Fig. 3 Soil and physiographic data of the collection sites in the Potohar region.

Table 1 Eco-morphological and physiological characteristics of *Chrysopogon serrulatus* collected from different altitudinal ranges in the Potohar region (means±SE)

Parameters		400 m	600 m	800 m	1000 m	1200 m	1400 m	LSD
Ecological	RD	30.8±3.5b	15.1±1.1d	21.3±1.8c	37.7±4.1a	22.3±2.5c	1.9±0.3e	4.9
	RF	26.6±3.9a	9.4±1.3c	8.4±1.1c	11.7±1.4b	10.2b±1.2c	1.3±0.1d	3.1
	RC	19.9±2.2ab	21.6±2.4a	20.4±1.4a	19.5±2.5ab	17.2±1.8b	0.7±0.03c	2.7
	IV	77.3±6.4a	46.1±5.2c	49.9±2.9c	68.9±4.7b	49.7±5.2c	3.9±0.4d	6.2
Morphological	PH	82.3±9.8b	88.3±5.2a	77.1±5.7c	71.5±5.4d	41.6±3.9e	32.5±3.3f	3.6
	LN	46.9±3.2a	39.7±4.6b	37.1±4.1b	25.9±3.4c	21.6±2.2c	16.2±2.1d	4.3
	LA	562.9±62.7b	634.0±72.1a	622.5±55.8a	542.6±54.7b	441.5±49.5c	383.1±44.0d	25.3
	SF	43.4±3.9a	44.4±3.2a	35.6±3.3b	20.1±3.1c	9.9±1.3d	9.0±0.8d	4.4
	SD	12.5±2.1a	13.8±1.4a	12.9±1.1a	7.7±0.8b	4.3±0.2c	3.0±0.1c	1.6
Physiological	Ca	1.7±0.2bc	2.4±0.3a	2.1±0.2ab	1.5±0.1c	1.3±0.2c	0.7±0.1d	0.4
	Cb	0.9±0.1a	0.9±0.0a	0.7±0.1b	0.7±0.0c	0.6±0.0d	0.44±0.0e	0.1
	Cr	0.056±0.001b	0.063±0.002a	0.062±0.004a	0.054±0.003b	0.044±0.004c	0.038±0.002d	0.003
	K^+	8.5±0.7c	11.6±b0.9	18.2±1.1a	17.5±1.2a	12.6±1.1b	6.3±0.5d	2.7
	Ca^{2+}	10.7±0.8e	18.6±0.9c	28.7±1.9a	21.6±1.4b	13.71.2±d	9.6±0.8e	1.5

Note: Mean values sharing different letters (a, b, c, d, e, f) are statistically significant at $p<0.05$.
Abbreviations: SE–Standard error. Ecological parameters: RD–Relative density, RF–Relative frequency, RC–Relative cover, IV–Importance value. Morphological parameters: PH–Plant height (cm), LN–Leaves per plant, LA–Total leaf area (cm^2), SF–Shoot fresh weight (g $plant^{-1}$), SD–Shoot dry weight (g $plant^{-1}$). Physiological parameters: Ca–Chlorophyll a (mg g^{-1} f. wt.), Cb–Chlorophyll b (mg g^{-1} f. wt.), Cr–Carotenoids (mg g^{-1} f. wt.), K^+–Shoot K^+ (mg g^{-1} d. wt.), Ca^{2+}–Shoot Ca^{2+} (mg g^{-1} d. wt.).

3.5 Root anatomy

Root anatomical traits such as epidermal cell area, cortical region thickness and cortical cell area, metaxylem area and pith cell area decreased with increasing elevation, while pith area and metaxylem vessel number increased (Table 2, Fig. 4). Sclerification of the outer cortical region was only found at 600 m elevation. Tissues outside the endodermis (cortex and epidermis) were completely absent at 1400 m.

3.6 Stem anatomy

Among stem anatomical characteristics, cortical cell area decreased consistently with elevation. Stem anatomical characteristics such as stem cross-sectional area, epidermal cell area, sclerenchyma thickness, vascular bundle number and area, and metaxylem area were the highest at intermediate elevations. At the highest elevation, most stem anatomical characteristics were statistically less (Table 2, Fig. 4).

3.7 Leaf sheath anatomy

Leaf sheath thickness and cortical cell area increased with the increase in elevation up to 1000 m elevation (Table 2, Fig. 4). These traits decreased at higher elevations. The highest epidermal cell area (135.4 μm^2), vascular bundle area (13245.7 μm^2) and metaxylem area (115.4 μm^2) were recorded at 800 m elevation. Sclerenchymatous thickness outside vascular bundles was the maximum (65.2 µm) at the highest elevation, where less soil water. Precipitation increased with elevation; steep slopes mean thinner soils and more runoff, therefore, less soil water. The minimum leaf sheath thickness (247.8 µm), epidermal cell area (92.1 μm^2), cortical cell area (1125.8 μm^2), sclerenchymatous thickness (12.1 µm) and metaxylem area (85.4 μm^2) were observed at 400 m (Table 2, Fig. 4), where mountain slopes was the minimum.

3.8 Leaf blade anatomy

Leaf anatomical traits showed no specific pattern along elevation gradient. Leaf traits such as epidermal cell area, bundle sheath area and metaxylem area were the maximum at 1200 m (Table 2, Fig. 4). Midrib thickness was the maximum (556.4 µm) at 400 m and the minimum (171.8 µm) at 1000 m, while the thickest lamina (403.6 µm) was recorded at 1200 m and the thinnest at 1000 m (119.8 µm). Trichome number, trichome length, vascular bundle area and sclerenchymatous thickness markedly increased at higher elevations (1200 and 1400 m), where slope was the maximum. Stomatal density was the maximum (146.1 per mm^2) at 1000 m and the minimum (25.1 per mm^2) at 400 m, while stomatal area was the

Table 2 Root, stem and leaf anatomical characteristics of *Chrysopogon serrulatus* collected from different altitudinal ranges in the Potohar region (means±SE)

Anatomical characteristics		400 m	600 m	800 m	1000 m	1200 m	1400 m	LSD
Root	RA	2.21±0.3b	3.14±0.4a	1.19±0.2c	1.21±0.2c	1.12±0.1d	3.15±0.2a	0.32
	REA	964.1±101.3a	215.7±32.8b	173.3±19.2c	--	--	--	25.9
	RCT	205.3±22.4a	190.7±21.6b	109.7±12.7c	--	--	--	7.3
	RCA	1541.7±166.4a	581.6±62.5b	201.7±22.1c	132.9±11.8d	120.5±1.5d	--	15.8
	RST	--	47.6±4.5a	--	--	--	--	--
	RVA	1.03±0.11b	0.65±0.06d	0.64±0.06d	1.08±0.1b	0.84±0.08c	2.96±0.3a	1.2
	RMA	1823.4±181.2a	521.6±51.3c	519.3±51.7c	632.1±62.1b	629.7±69.7b	192.6±12.4d	5.6
	RMN	9.4±0.9d	14.9±1.3b	12.3±1.4c	16.5±1.4b	16.3±1.4b	24.5±3.2a	3.7
	RPA	3472.1±348.3f	4761.8±475.3d	4417.1±439.3e	8321.5±833.2b	7419.6±777.3c	10321.5±972.1a	64.8
	RPC	472.5±46.9a	207.8±20.5b	204.6±21.2b	121.5±12.1d	131.9±11.6c	115.4±9.3d	9.4
Stem	SA	7.5±0.6d	11.2±0.9c	14.9±1.1a	12.4±0.8b	3.5±0.2e	4.1±0.5e	0.8
	SEA	31.2±2.8ab	34.2±3.1a	33.8±2.9a	34.3±2.2a	27.6±b3.2c	24.5±2.8c	3.7
	SCA	8186.5±800.4a	7654.3±657.8b	6547.1±666.9c	4558.9±521.3d	3564.1±229.4e	3397.4±237.7e	201.5
	SST	47.2±4.3de	77.1±6.1c	151.4±12.9b	201.7±22.0a	45.1±3.8e	56.7±4.7d	4.7
	SVN	21.7±2.5b	22.7±2.1b	31.2±4.3a	21.9±2.7b	14.6±1.9c	11.4±1.3d	2.1
	SVA	12436.8±1117.1d	20179.6±2029.2b	19547.6±1951.2c	35412.4±2298.1a	12455.7±1045.0d	9654.3±1011.3e	506.1
	SMA	97.3±8.4b	167.5±13.2a	166.4±15.6a	167.4±12.4a	56.5±6.2c	47.2±5.3c	10.3
Leaf sheath	HT	247.8±35.2e	339.7±32.1d	445.7±49.6b	501.2±44.9a	256.1±26.3e	385.4±33.5c	10.5
	HEA	92.1±7.8c	121.4±13.6b	135.4±10.4a	114.6±12.8b	93.2±7.5c	87.4±8.8c	11.4
	HCA	1125.8±115.3e	2356.7±245.7c	3256.9±322.1b	6142.8±549.3a	1423.6±109.8d	3258.7±344.7b	26.8
	HST	12.1±1.1c	33.5±2.1b	56.7±6.4a	25.1±1.3b	15.7±1.1c	65.2±5.4a	7.4
	HVA	4568.7±435.1e	8563.7±651.4b	13245.7±1011.4a	7456.5±564.0c	5966.3±678.2d	3524.7±430.1f	131.7
	HMA	85.4±6.5c	102.3±9.2b	115.4±9.9a	101.4±11.7b	99.7±8.3b	97.6±9.6b	5.2
Leaf blade	LMT	556.4±62.5a	463.1±52.1c	468.3±44.9c	171.8±21.6e	506.1±46.7b	271.5±33.8d	11.7
	LLT	132.8±14.2c	141.6±15.9c	135.4±14.5c	119.8±12.6d	403.6±35.4a	252.1±32.7b	11.3
	LEA	165.3±22.6c	146.8±19.5d	174.6±16.3c	102.6±9.8e	401.8±37.1a	311.5±30.9b	13.6
	LST	28.5±3.1bc	21.2±2.4c	31.5±3.6b	12.4±1.1d	51.2±4.3a	35.6±4.7b	12.5
	LBS	93.4±8.8c	114.6±12.3b	82.5±7.1d	66.2±5.4e	123.4±13.7a	97.6±8.2c	7.1
	LVA	3125.8±225.6c	2544.7±189.3d	2645.7±253.8d	1124.8±128.9e	6568.7±556.7a	4413.5±432.7b	104.3
	LMA	68.2±5.4b	66.4±5.5b	82.5±7.2a	35.7±4.3c	83.2±7.2a	65.9±5.4b	3.7
	LTN	4.1±0.5c	3.6±0.3c	3.8±0.2c	3.2±0.4c	10.5±0.8a	9.4±0.7b	2.6
	LTL	11.2±0.9b	10.9±0.8b	8.3±0.7c	9.2±0.6c	13.4±0.8a	13.5±0.7a	2.2
	LSD	25.1±3.2d	66.9±4.7c	139.3±8.9a	146.1±10.5a	119.3±12.4b	69.7±5.6c	7.4
	LSA	273.4±24.6b	463.1±44.7a	268.3±31.2b	271.5±26.9b	171.8±15.4c	167.5±14.9c	5.8

Note: Mean values sharing different letters (a, b, c, d, e) are statistically significant at $p<0.05$, SE–Standard error.

Abbreviations: RA–Root cross-sectional area (mm²), REA–Epidermal cell area (µm²), RCT–Cortical region thickness (µm), RCA–Cortical cell area (µm²), RST–Sclerenchymatous thickness (µm), RVA–Stelar area (mm²), RMA–Metaxylem area (µm²), RMN–Metaxylem vessel number, RPA–Pith area (µm²), RPC–Pith cell area (µm²). SA–Stem area (mm²), SEA–Epidermal cell area (µm²), SCA–Cortical cell area (µm²), SST–Sclerenchymatous thickness (µm²), SVN–Vascular bundle number, SVA–Vascular bundle area (µm²), SMA–Metaxylem area (µm²). HT–Sheath thickness (µm), HEA–Epidermal cell area (µm²), HCA–Cortical cell area (µm²), HST–clerenchymatous thickness (µm), HVA–Vascular bundle area (µm²), HMA–Metaxylem area (µm²). LMT–Midrib thickness (µm), LLT–Lamina thickness (µm), LEA–Epidermal cell area (µm²), LST–Sclerenchymatous thickness (µm²), LBS–Bundle sheath cell area (µm²), LVA–Vascular bundle area (µm²), LMA–Metaxylem vessel area (µm²), LTN–Trichome number per mm², LTL–Trichome length (µm), LSD–Stomatal density per mm², LSA–Stomatal area (µm²).

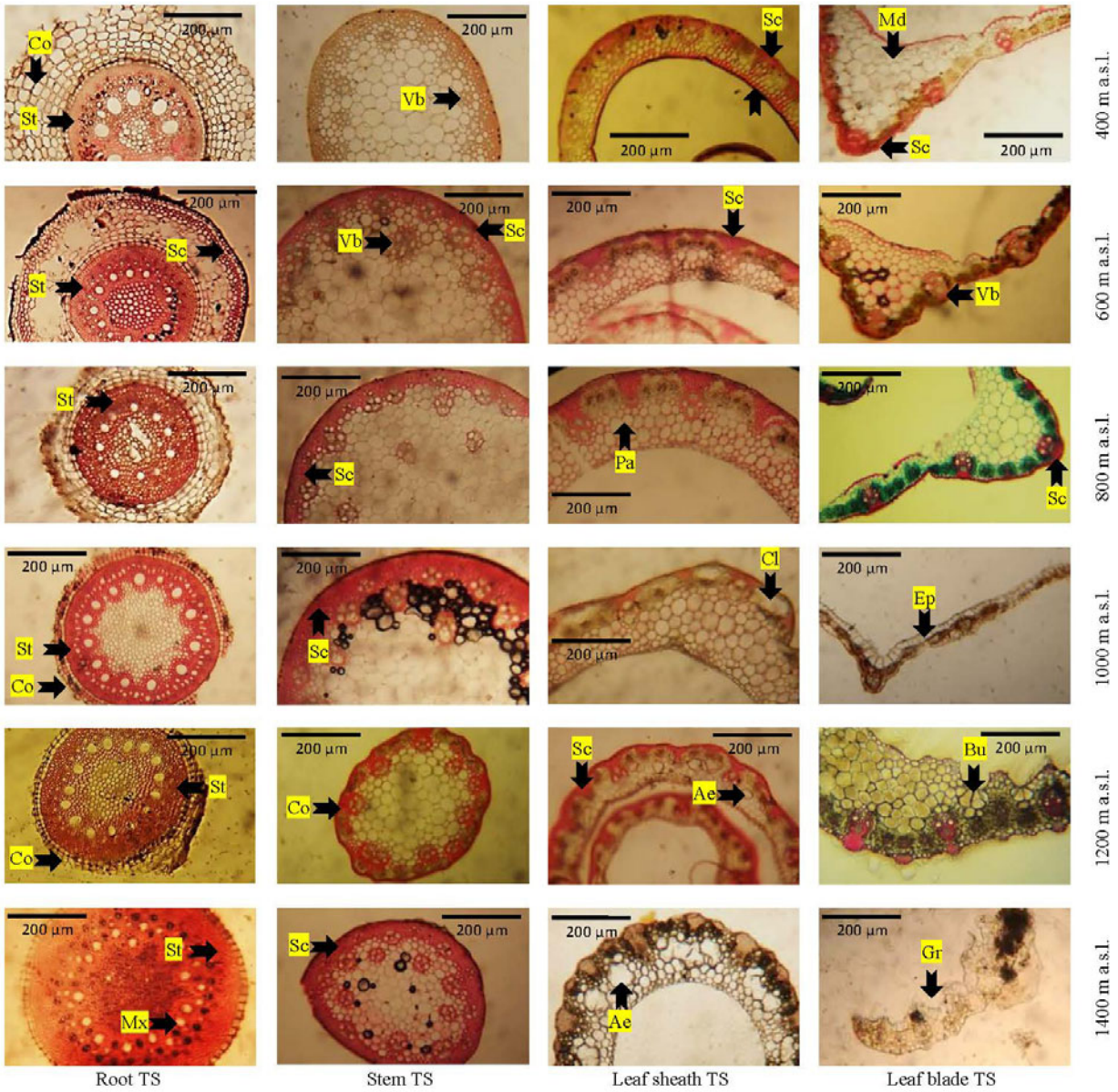


Fig. 4 Root, stem and leaf transverse sections of *Chrysopogon serrulatus* collected from different altitudinal ranges in the Potohar region. Ae–Aerenchyma, Bu–Bulliform cells, Cl–Chlorenchyma, Co–Cortex, Ep–Epidermis, Gr–Groove, Md–Midrib. Mx–Metaxylem, Pa–Parenchyma, Sc–Sclerenchyma, St–Stele, Vb–Vascular bundle. Scale bar given in all transverse sections (resolution of all sections is 20X).

highest 463.1 µm²) at 600 m and the lowest (167.5 µm²) at 1400 m (Table 2, Fig. 5).

3.9 Specific anatomical modifications

In roots, the proportion of cortical parenchyma decreased, while area of stelar region and metaxylem vessels (size and/or density) increased at the highest elevation. The cortical region was not present at the highest elevation (1400 m). Sclerification in the vascular region increased significantly at the highest elevation (Fig. 4). Stem cross-sectional area decreased greatly at the higher elevations (1200 and 1400 m), whereas sclerification inside epidermis and outside vascular bundles increased significantly at 1000 m and above. Vascular bundles were close to the stem periphery at higher elevations. In leaf sheath, aerenchymatous cavities in parenchymatous region appeared at 1200 m elevation, while aerenchyma cell size increased markedly at the highest elevation. A unique feature of leaf anatomy was the increase in proportion of parenchymatous tissue and size/shape

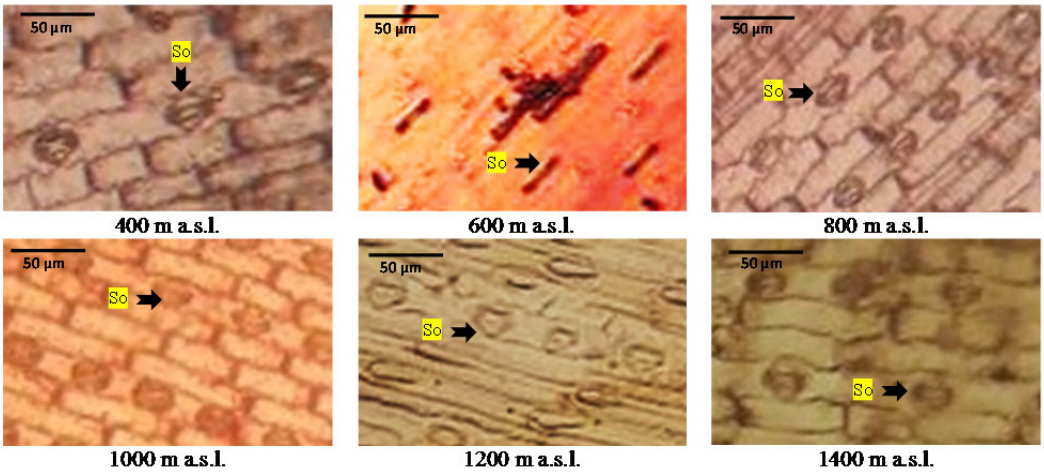


Fig. 5 Adaxial epidermal surface view of *Chrysopogon serrulatus* collected from different altitudinal ranges in the Potohar region. Scale bar given in all transverse sections (resolution of all sections is 40X). So–Stomata.

of motor cells at 1400 m elevation, which were fan shaped. Leaf width was extremely reduced at the highest elevation (Fig. 4).

3.10 Relationship of morphological, eco-physiological and anatomical traits

PCA biplots showing relationship among morphological, ecological, physiological and anatomical characteristics are presented in Fig. 6. The lowest elevation (400 m) was associated with root traits such as cell area of epidermis, cortex and pith, metaxylem area and cortical region thickness. Soil and environmental factors such as maximum and minimum temperatures, soil ECe, Na^+, K^+ and Cl^- showed association with 600 m elevation. Ecological traits such as relative cover and importance value, morphological traits such as plant height, leaves per plant, leaf area and shoot fresh and dry weights, physiological traits such as chlorophyll *a*, *b* and carotenoids, and stem cortical cell area were also linked to 600 m. Soil Ca^{2+}, shoot Ca^{2+} and K^+, leaf sheath epidermal area and vascular bundle area, and stem anatomical traits such as stem area, epidermal area, metaxylem area, number of vascular bundles were correlated with 800 m. Shoot K^+, stem sclerenchyma thickness and vascular bundle area, leaf sheath thickness, cortical cell area and metaxylem area, and leaf stomatal density showed close association with 100 m. Elevation 1200 m was associated with soil Mg^{2+} and most of the leaf anatomical traits, i.e., lamina thickness, epidermal cell area, sclerenchymatous thickness, vascular bundle area, trichome number and length. Average rainfall and root anatomical traits such as vascular bundle area, number of metaxylem vessels and pith area were linked to 1400 m elevation.

3.11 Relationship of soil and environmental traits with plant structural and functional traits

Correlation between soil and environmental traits with plant structural and functional traits are presented in Table 3. The maximum and minimum mean annual temperatures showed positive correlations with relative cover and importance value of *C. serrulatus* populations. Annual was rainfall negatively correlated with relative frequency ($p<0.001$), relative cover ($p<0.01$) and importance value ($p<0.05$). Soil Mg^{2+} was negatively correlated with relative density, relative cover and importance value ($p<0.05$). Annual rainfall negatively correlated ($p<0.05$) with plant height, leaf number and area, and shoot fresh and dry weights (Table 3). Soil ECe, Na^+ and Cl^- positively correlated with all morphological traits (plant height, leaf number and area, and shoot fresh and dry weights). A positive correlation ($p<0.05$) of soil K^+ was recorded with plant height and total leaf area. Among physiological traits, chlorophyll *a* was positively correlated with mean annual minimum temperature ($p<0.05$), soil K^+ ($p<0.01$) and shoot Cl^- ($p<0.05$), whereas negatively correlated with annual rainfall ($p<0.05$). Chlorophyll b was positively correlated with mean annual maximum and minimum temperatures ($p<0.05$), soil ECe, Na^+ ($p<0.01$), K^+ and Cl^- ($p<0.05$), while negatively correlated with annual rainfall ($p<0.01$). A negative

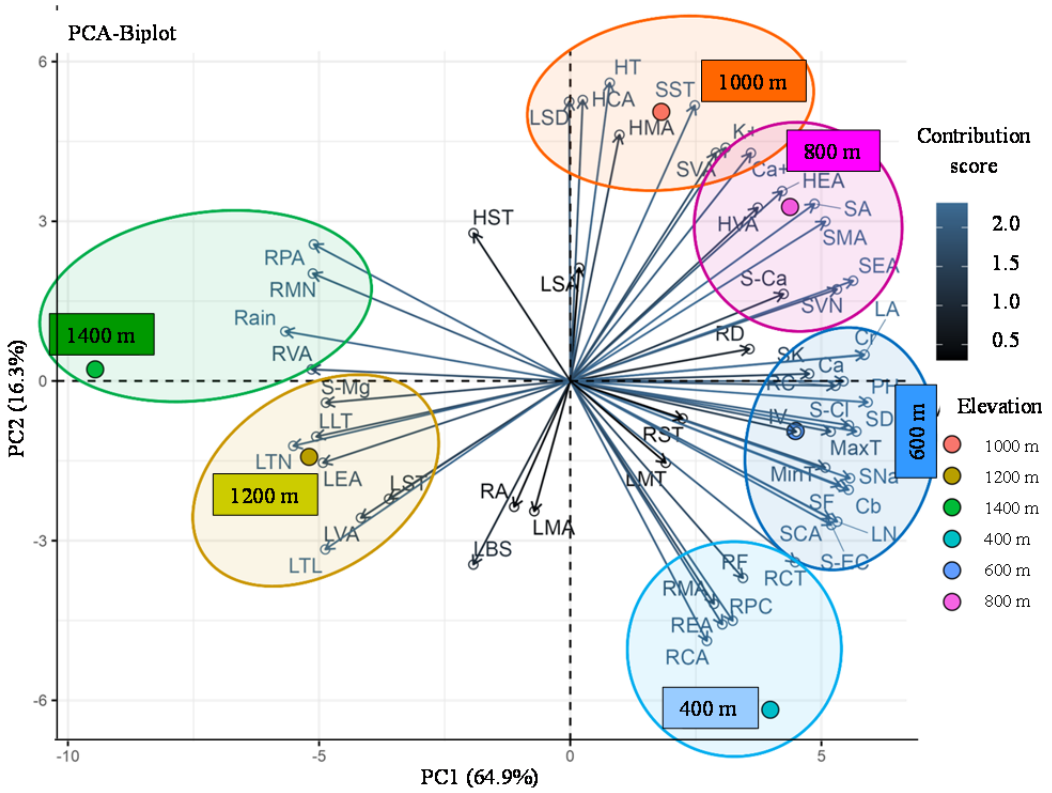


Fig. 6 Principal component analysis showing the relationship of morphological and eco-physiological characteristics anatomical attributes of *Chrysopogon serrulatus* along elevation gradient.

Environmental and soil physicochemical traits: MaxT- Maximum temperature, MinT- Minimum temperature, Rain- Annual rainfall, S-EC- Soil ECe, SNa- Soil Na^+, SK- Soil K^+, S-Ca- Soil Ca^{2+}, S-Mg-Soil Mg^{2+}, S-Cl- Soil Cl^-; **Ecology:** IV- Importance value, RC- Relative cover, RD- Relative density, RF- Relative frequency; **Leaf blade anatomy:** LBS- Bundle sheath cell area, LEA- Epidermal cell area, LLT Lamina thickness LMA- Metaxylem area,LMT- Midrib thickness, LSA- Stomatal area, LSD- Stomatal density, LST- Sclerenchymatous thickness, LTL- Trichome length, LTN- Trichome density, LVA- Vascular bundle area; **Leaf sheath anatomy:** HCA- Cortical cell area, HEA- Epidermal cell area, HMA- Metaxylem area, HST- Sclerenchymatous thickness, HT- Sheath thickness, HVA- Vascular bundle area; **Morphology:** LA- Leaf area, LN- Leaves per plant, PH- Plant height, SD- Shoot dry weight, SF- Shoot fresh weight; **Physiology:** Cr- Carotenoids, Ca- Chlorophyll *a*, Ca^+- Shoot Ca^{2+}, Cb- Chlorophyll *b*, K+- Shoot K^+; **Root anatomy:** RA- Root area, RCA- Cortical cell area, RCT- Cortical region thickness, REA- Epidermal cell area, RMA- Metaxylem area, RMN- Metaxylem vessel number, RPA- Pith area, RPC- Pith cell area, RST- Sclerenchymatous area, RVA- Vascular bundle area; **Stem anatomy:** SA- Stem area, SCA- Cortical cell area, SEA- Epidermal cell area, SMA- Metaxylem area, SST- Sclerenchymatous thickness, SVA- Vascular bundle area, SVN- Vascular bundle number.

correlation of carotenoids was recorded with annual rainfall ($p<0.01$), while Soil ECe, Na^+, K^+ ($p<0.05$) and Cl^- ($p<0.01$) positively correlated with carotenoids (Table 3).

The maximum and minimum annual mean temperatures was negatively correlated with root anatomical traits such as stelar area ($p<0.01$), metaxylem vessel number and pith area ($p<0.05$). Annual rainfall was positively correlated with stelar area, metaxylem vessel number ($p<0.01$) and pith area ($p<0.05$). Soil ECe was positively correlated with epidermal cell area ($p<0.05$), cortical region thickness ($p<0.01$) and pith cell area ($p<0.05$), while negatively correlated with metaxylem vessel number ($p<0.05$) and pith area ($p<0.01$). A positive correlation of soil Na^+ was observed with cortical region thickness and anegative correlation was recorded with metaxylem vessel number ($p<0.05$) and pith area ($p<0.01$). Soil K^+ was positively correlated with sclerenchymatous thickness ($p<0.05$). Soil Cl^- was positively correlated

Table 3 Pearson's correlation coefficients showing correlation of soil and environmental traits with structural and functional traits of *Chrysopogon serrulatus* along elevation gradient

Characteristics		MaxT	MinT	Rain	Soil ECe	Soil Na^+	Soil K^+	Soil Ca^{2+}	Soil Mg^{2+}	Soil Cl^-
Ecology	Relative density	0.737	0.626	-0.688	0.344	0.343	0.221	0.692	-0.861*	0.293
	Relative frequency	0.683	0.661	-0.989***	0.721	0.612	0.192	0.455	-0.639	0.484
	Relative cover	0.988***	0.968**	-0.969**	0.654	0.732	0.754	0.636	-0.829*	0.698
	Importance value	0.890*	0.822*	-0.862*	0.607	0.594	0.405	0.682	-0.888*	0.517
Morphology	Plant height	0.806	0.802	-0.902*	0.898*	0.955**	0.832*	0.582	-0.801	0.916*
	Leaf number	0.765	0.794	-0.863*	0.993***	0.976***	0.652	0.535	-0.603	0.930**
	Total leaf area	0.797	0.800	-0.887*	0.817*	0.912*	0.868*	0.627	-0.699	0.934**
	Shoot fresh weight	0.694	0.736	-0.815*	0.979***	0.996***	0.766	0.448	-0.575	0.956**
	Shoot dry weight	0.748	0.775	-0.862*	0.934**	0.983***	0.807	0.564	-0.607	0.986***
Physiology	Chlorophyll *a*	0.808	0.844*	-0.871*	0.769	0.879	0.934**	0.475	-0.612	0.881*
	Chlorophyll *b*	0.877*	0.907*	-0.936**	0.928**	0.959**	0.832*	0.447	-0.752	0.867*
	Carotenoids	0.800	0.802	-0.890*	0.816*	0.911*	0.867*	0.631	-0.700	0.934**
	Shoot K^+	0.521	0.427	-0.496	0.033	0.173	0.358	0.727	-0.497	0.320
	Shoot Ca^{2+}	0.445	0.386	-0.487	0.188	0.343	0.485	0.706	-0.369	0.529
Root anatomy	Root area	-0.439	-0.331	0.333	0.164	0.131	0.134	-0.681	0.311	0.011
	Epidermal cell area	0.458	0.481	-0.535	0.821*	0.687	0.154	0.321	-0.364	0.589
	Cortical region thickness	0.592	0.666	-0.704	0.963**	0.941**	0.664	0.259	-0.411	0.867*
	Cortical cell area	0.409	0.446	-0.483	0.801	0.668	0.193	0.164	-0.364	0.525
	Sclerenchymatous thickness	0.271	0.375	-0.315	0.391	0.503	0.838*	-0.351	-0.242	0.380
	Stelar area	-0.961**	-0.965**	0.949**	-0.657	-0.744	-0.775	-0.616	0.694	-0.743
	Metaxylem area	0.570	0.556	-0.586	0.697	0.562	0.078	0.395	-0.510	0.441
	Metaxylem vessel number	-0.918**	-0.907*	0.941**	-0.831*	-0.817*	-0.520	-0.730	0.677	-0.802
	Pith area	-0.824*	-0.864*	0.888*	-0.921**	-0.920**	-0.643	-0.573	0.512	-0.911*
	Pith cell area	0.498	0.522	-0.574	0.842*	0.715	0.185	0.360	-0.373	0.627
Stem anatomy	Stem area	0.523	0.465	-0.626	0.492	0.618	0.630	0.731	-0.587	0.739
	Epidermal cell area	0.796	0.751	-0.854*	0.643	0.758	0.810	0.673	-0.850*	0.769
	Cortical cell area	0.685	0.729	-0.802	0.995***	0.987***	0.701	0.431	-0.553	0.934*
	Sclerenchymatous thickness	0.219	0.092	-0.254	-0.035	0.081	0.205	0.623	-0.503	0.205
	Vascular bundle number	0.684	0.647	-0.777	0.660	0.747	0.608	0.854*	-0.546	0.880*
	Vascular bundle area	0.377	0.265	-0.379	0.044	0.166	0.405	0.449	-0.731	0.174
	Metaxylem area	0.609	0.564	-0.691	0.518	0.659	0.782	0.600	-0.716	0.711
Leaf anatomy	Midrib thickness	0.122	0.252	-0.215	0.472	0.546	0.712	-0.342	0.003	0.475
	Lamina thickness	-0.476	-0.429	0.626	-0.744	-0.783	-0.580	-0.602	0.688	-0.796
	Epidermal cell area	-0.487	-0.427	0.615	-0.663	-0.718	-0.603	-0.556	0.768	-0.705
	Sclerenchymatous thickness	-0.264	-0.197	0.375	-0.422	-0.486	-0.502	-0.311	0.700	-0.436
	Bundle sheath cell area	-0.025	0.115	0.114	-0.092	-0.097	0.118	-0.642	0.397	-0.207
	Vascular bundle area	-0.322	-0.246	0.452	-0.490	-0.557	-0.491	-0.514	0.679	-0.572
	Metaxylem area	0.043	0.144	-0.020	0.070	0.048	-0.061	-0.023	0.540	0.150
	Trichome number	-0.626	-0.577	0.747	-0.754	-0.816*	-0.686	-0.651	0.795	-0.819*
	Trichome length	-0.563	-0.480	0.654	-0.492	-0.588	-0.506	-0.855*	0.635	-0.717
	Stomatal density	0.075	-0.021	0.001	-0.489	-0.354	-0.030	0.393	-0.095	-0.184
	Stomatal area	-0.155	-0.070	0.108	-0.077	0.082	0.542	-0.384	0.195	0.114
Leaf sheath anatomy	Leaf sheath thickness	-0.180	-0.283	0.111	-0.239	-0.125	0.041	0.350	-0.157	0.029
	Epidermal cell area	0.487	0.463	-0.560	0.358	0.522	0.682	0.603	-0.407	0.670
	Parenchymatous cell area	-0.177	-0.299	0.150	-0.336	-0.242	-0.030	0.233	-0.283	-0.167
	Sclerenchymatous thickness	-0.680	-0.662	0.567	-0.313	-0.266	-0.231	-0.134	0.652	-0.068
	Vascular bundle area	0.483	0.462	-0.533	0.293	0.441	0.546	0.668	-0.265	0.633
	Metaxylem area	0.039	0.021	-0.058	-0.197	-0.030	0.260	0.339	0.118	0.196

Notes: * Significant at $p<0.05$, ** Significant at $p<0.01$, *** Significant at $p<0.001$. MaxT-Maximum temperature; MinT-Minimum temperature; Rain-Annual rainfall.

with cortical region thickness ($p<0.05$), but negatively correlated with pith area ($p<0.05$) (Table 3). Stem epidermal cell area was negatively correlated with annual rainfall and soil Mg^{2+} ($p<0.05$). Cortical cell area was positively correlated with soil ECe, Na^+ ($p<0.001$) and Cl^- ($p<0.05$). Vascular bundle number was positively correlated with soil Ca^{2+} and Cl^- ($p<0.05$). Among leaf anatomical traits, trichome number was negatively correlated with soil Na^+ and Cl^- ($p<0.05$), while trichome length showed a negative correlation with soil Ca^{2+} ($p<0.05$) (Table 3).

3.12 Magnitude of soil physicochemical, plant morpho-physiological and anatomical traits

Path coefficient analysis between environmental and ecological traits presented a strong negative

correlation of annual rainfall with relative density, relative cover and importance value (Fig. 7a). Elevation negatively correlated with relative frequency while positively correlated with relative density. Among soil physicochemical and ecological traits, soil Cl^- showed a strong negative and soil Na^+ a strong positive correlation with relative density, relative frequency and importance value (Fig. 7b). Root pith cell area correlated strongly and positively with plant height, leaf area and leaf number (Fig. 7c). A strong negative association of root cortical cell area was observed with plant height and leaf number, while leaf area negatively correlated with root vascular bundle area and metaxylem area. Among stem anatomical traits, stem area positively correlated with leaf area and shoot fresh and dry weights, whereas negatively correlated with leaves per plant (Fig. 7d). The stem epidermal area was positively

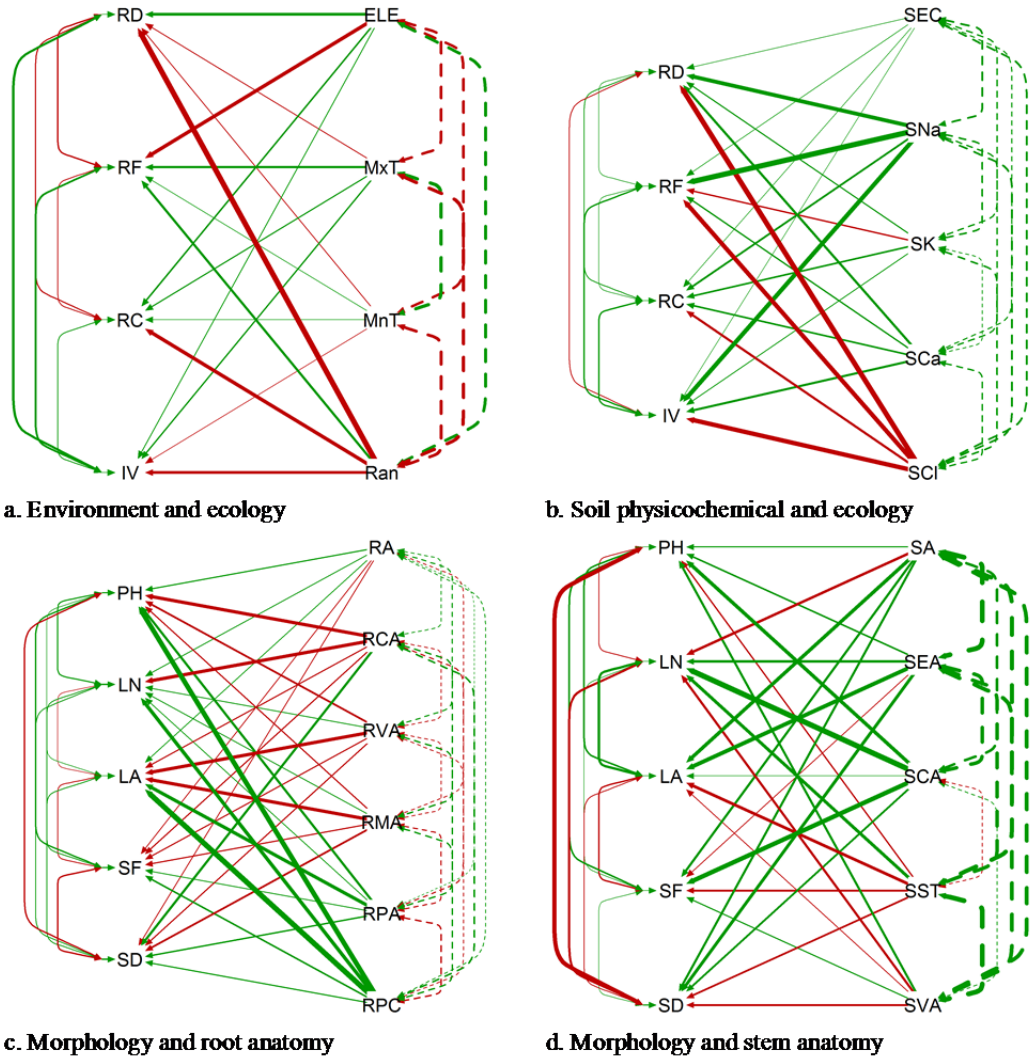
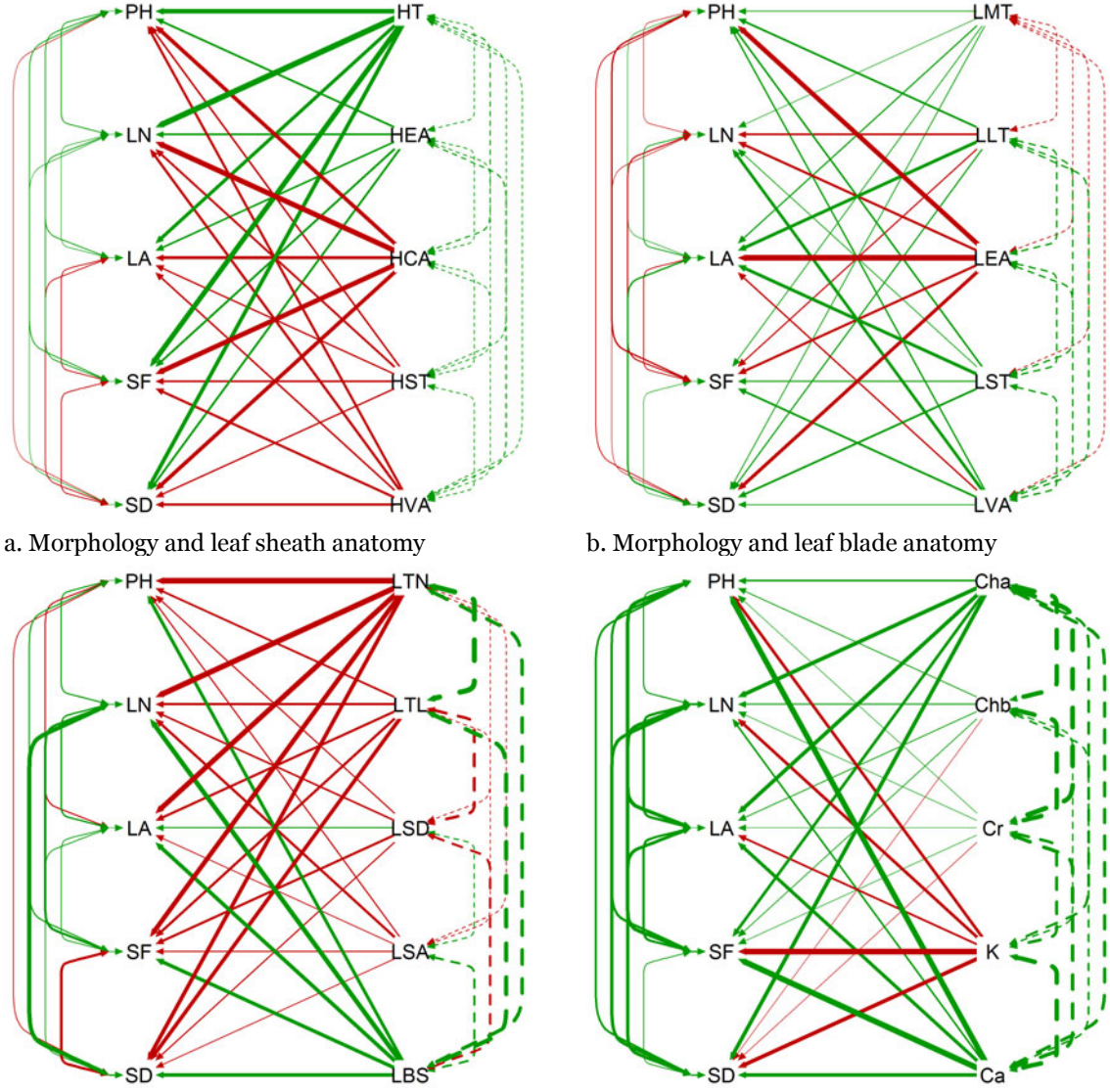


Fig. 7 Path coefficient analysis for a) environment and ecology, b) soil physicochemical and ecology, c) morphology and root anatomy, and d) morphology and stem anatomy of *Chrysopogon serrulatus* along elevation gradient. The traits with high variance were omitted from dataset. The strength of correlation is indicated by thickness of arrows. The green arrows indicate +ve while red arrows indicate –ve correlation.

Ecology: IV- Importance value, RC- Relative cover, RD- Relative density, RF- Relative frequency; **Environmental traits:** ELE- Elevation, MxT- Maximum temperature, MnT- Minimum temperature, Ran- Annual rainfall; **Soil physicochemical traits:** SEC- Soil ECe, SNa- Soil Na^+, SK- Soil K^+, SCa- Soil Ca^{2+}, SCl- Soil Cl^-, Morphology: LA- Leaf area, LN- Leaves per plant, PH- Plant height, SD- Shoot dry weight, SF- Shoot fresh weight; **Root anatomy:** RA- Root area, RCA- Cortical cell area, RMA- Metaxylem area, RPA- Pith area, RPC- Pith cell area, RVA- Vascular bundle area; **Stem anatomy:** SA- Stem area, SCA- Cortical cell area, SEA- Epidermal cell area, SST- Sclerenchymatous thickness, SVA- Vascular bundle area.

correlated with leaf number and area and shoot fresh weight. Cortical cell area positively correlated with plant height, leaf number and shoot fresh weight. Sclerenchymatous thickness negatively correlated with leaf area, shoot fresh and dry weights, while vascular bundle area negatively correlated with leaf number and shoot dry weight.

A strong positive correlation of morphological traits was observed with leaf sheath thickness, while negatively correlated with cortical cell area (Fig. 8a). Among leaf anatomical characteristics, epidermal thickness strongly and negatively correlated with plant height, leaf area and shoot dry weight (Fig. 8b). A positive association of lamina thickness was



a. Morphology and leaf sheath anatomy

b. Morphology and leaf blade anatomy

c. Morphology and leaf epidermal traits

d. Morphology and physiology

Fig. 8 Path coefficient analysis for a) morphology and leaf sheath anatomy, b) morphology and leaf blade, c) morphology and leaf epidermis, and d) morphology and physiology of *Chrysopogon serrulatus* along elevation gradient. The traits with high variance were omitted from dataset. The strength of correlation is indicated by thickness of arrows. The green arrows indicate +ve while red arrows indicate –ve correlation.

Morphology: LA- Leaf area, LN- Leaves per plant, PH- Plant height, SD- Shoot dry weight, SF- Shoot fresh weight; **Leaf sheath anatomy:** HCA- Cortical cell area, HEA- Epidermal cell area, HST- Sclerenchymatous thickness, HT- Sheath thickness, HVA- Vascular bundle area; **Leaf blade anatomy:** LEA- Epidermal cell area, LLT Lamina thickness, LMT- Midrib thickness, LST- Sclerenchymatous thickness, LVA- Vascular bundle area; **Leaf epidermal traits:** LBS- Bundle sheath cell area, LSA- Stomatal area, LSD- Stomatal density, LTL- Trichome length, LTN- Trichome density; **Physiology:** Cr- Carotenoids, Cha- Chlorophyll *a*, Ca- Shoot Ca^{2+}, Chb- Chlorophyll *b*, K- Shoot K^{+}.

recorded with plant height and leaf area, while sclerenchymatous thickness positively correlated with leaf area and vascular bundle area with leaves per plant. Number of trichomes strongly negatively correlated with all morphological traits (Fig. 8c). Trichome length negatively associated with leaf number and area and shoot fresh and dry weights. Bundle sheath cell area positively correlated with morphological traits. Chlorophyll *a* positively correlated with leaf number and area and shoot fresh and dry weights (Fig. 8d). Shoot Ca^{2+} strongly and positively correlated with plant height and shoot fresh and dry weights. A strong negative correlation of shoot fresh weight was noted with shoot K^{+}.

3.13 Change in adaptive mechanisms along elevational gradient

Modifications based on anatomical features varied in different organs along elevations (Fig. 9). The cortical region was the thickest at 400 m, which was composed of regularly arranged and tightly packed parenchymatous cells. Aerenchyma developed at 600 m, but cortical region thickness was greatly reduced at 800 m. Cortical region, along with epidermis was absent at higher elevations. Sclerification of stelar region generally increased with increase in elevation. In root, mesomorphic traits changed to xeromorphic by increased sclerification in stele, while metaxylem number increased significantly and area reduced.

Stem area and proportion of cortical parenchyma consistently increased with increase in elevation up to 1000 m, and then abruptly decreased at higher elevations (Fig. 9). Sclerification around vascular region was the maximum at 1000 m. Vascular bundle area was significantly reduced at the highest elevation. The mechanism in stems shifted from mesomorphy at lower elevation to succulence up to 1000 m by development of high proportion of storage parenchyma and further switched to xeromorphy by increased sclerification and reduction of stem-cross-sectional area at highest elevation.

Sclerification outside vascular tissue in leaf sheath increased consistently up to 800 m, and thereafter decreased. At the highest elevation, sclerification thickness was the maximum (Table 2, Fig. 9). Formation of aerenchyma started at 1200 m, which was fully developed at 1400 m. At highest elevation, the xeromorphy (sclerification and high proportion of storage parenchyma) was changed to aerenchyma formation in parenchymatous region.

Midrib thickness decreased up to 1000 m in *C. serrulatus* (Fig. 9). Leaf thickness increased significantly at 1200 m, where high proportion of parenchymatous tissue was observed with increase in size of bulliform cells. Stomatal density and shape significantly changed with increase in elevation. Stomata shape changed from ovate to circular at lower elevation, but rhomboid at 1200 m and narrowly elliptic at 1400 m. The change noted was from mesomorphy to xeromorphy at moderate elevations and further to succulence at higher elevations.

4 Discussion

The air pressure gradient is too small to be significant over our study elevational gradient of 1000 m (i.e., from 400 to 1400 m). High temperatures over 40°C and low annual rainfall, especially at lower elevations (up to 800 m) are real drought and heat stress indicators. Perennial grass *C. serrulatus*, such as its counterpart in North America, deals well with drought and heat (Joyce et al. 2016). The allelochemicals reduce competition that led to their presence in bunches (Chuah et al. 2014). There are two unique features of *C. serrulatus*: (1) this plant does well at low elevations and can deal with saline soils, aridity, and hot temperatures, and in contrast, (2) it is present at elevations as high at 1400 m. Though it is not ecological as important, other grasses such as *Themeda anathera, Lolium perenne, Aristida cyanantha* and *Bromus* spp. out compete it (Hameed et al. 2012), but it does survive, and it demonstrates structural changes which may facilitate its presence and survival at 1,400 m.

Density, frequency and percent cover of *C. serrulatus* was more at lower elevations whereas species number and percent cover decreased significantly above 1000 m. Plant growth parameters such as height, leaves per plant, total leaf area, fresh and dry weights also decreased with increase in elevation. *C. serrulatus* is a species of mountainous regions where conditions are more or less xeric, but better growth, biomass production and distributional pattern at lower elevations indicates its sensitivity to low temperatures or wind or poor nutrients or shorter growth period at high elevations. Though it survived

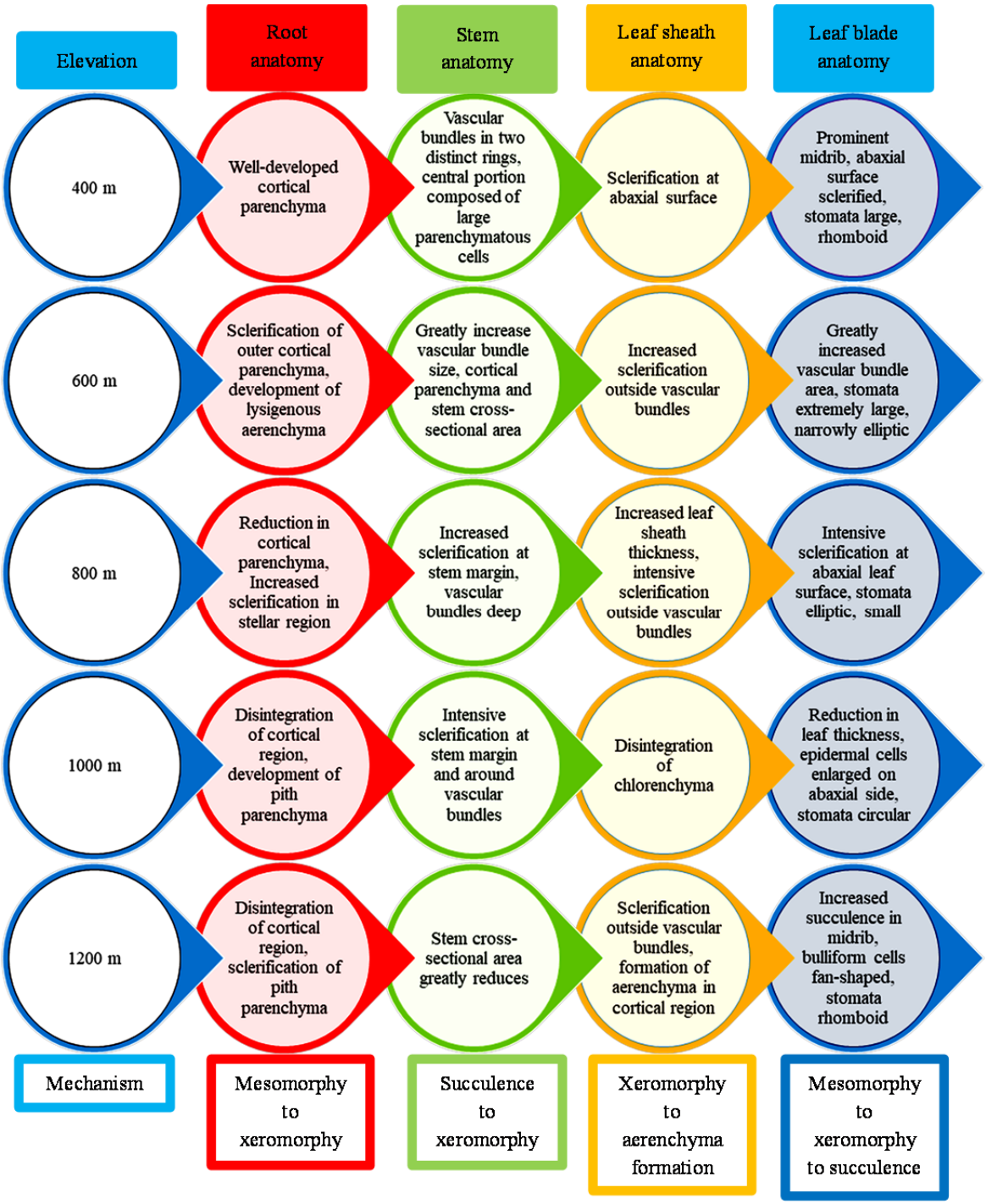


Fig. 9 Switching of anatomical mechanisms of *Chrysopogon serrulatus* along altitudinal gradient.

at higher elevations, all growth traits were severely affected. Stunted growth at higher elevations has earlier been reported by Tardella et al. (2017). This is a survival strategy of plants colonizing cool temperatures and all the other stress parameters, as it minimizes the exposure to external environments (Orwig and Abrams 1997). Chlorophyll and ion content (K^+ and Ca^{2+}) also decreased with elevation, and that also contributed to reduction in overall growth of *C. serrulatus* at high elevations (Wingler et al. 2015).

Rainfall increased exponentially with elevation, i.e., from 370 to 478 mm at low elevation to the maximum rainfall 1441 m as recorded at the highest

elevation (1400 m). Slopes of the hills increased greatly with increase in elevation, resulting in in an increase in xeromorphic conditions due to unavailability of rainwater. Here, water is mostly wasted through runoff and a little water is available in rooting zone of plants growing at the high elevations. Additionally, the temperature drops with increasing altitudes. These factors along with many other environmental constraints induce xeromorphic characters in plants enabling them to adopt these elevational gradients. Underground organs (roots) in *C. serrulatus* showed increased xeromorphy with elevational uplift, which included decreased proportion of parenchyma and increased sclerification, particularly in the stelar region (Moura et al. 2010). Such modification is critical for relatively sensitive species to survive in less favourable conditions of higher elevation (Scholz et al. 2012) by minimizing water loss from root surface (Rodrigues et al. 2021). Moreover, this protects roots from collapse under desiccated conditions (Zhang et al. 2020). Intensive sclerification was also recorded in the leaf sheath at the highest elevation. Leaf sheath covers the major proportion of stem in *C. serrulatus* (Husain et al. 2009), and increased sclerification protects aerial plant organs from collapse (Crang et al. 2018). Water conservation is an adapted strategy of a plant in drought-prone habitats. Metaxylem area and pith area responded positively to elevation as they increased at the highest elevation. This improves water conduction and provides better water storage capability (Kuster et al. 2018). Plant colonizing the higher elevations showed a peculiar modification in leaves, i.e., significant increase in trichome length. Such modification improves survival at high elevation by shielding plants from irradiance (Amada et al. 2020), cool winds (Sack and Buckley 2020) and water scarcity (Xiao et al. 2017). Moreover, decreased stomatal area is associated with reduced transpiration rate (Li et al. 2017), which is again a critical modification in water limited environments. Another prominent feature of the high elevation population was aerenchyma formation in leaf sheath and high proportion of storage parenchyma in leaf sheath and leaf blade. This is linked with increased succulence, a characteristic feature of many desert succulents (Zeng et al. 2018).

Older roots in perennial species such as *C. serrulatus* lose their softer tissues such as epidermis and cortical parenchyma due to soil friction, as was reported by Naz et al. (2013) in *Aeluropus lagopoides*, Naz et al. (2015) in *Lasiurus scindicus*, Fatima et al. (2021) in *Cymbopogon jwarancusa*, and Iqbal et al. (2022) in *Cynodon dactylon*. Leaf blades were more responsive to environmental conditions in plants colonizing 1200 m. Environmental and soil conditions at 1200 m elevation suited leaf anatomical traits, where the maximum value of midrib and lamina thicknesses, cell area of epidermis and bundle sheath, vascular bundle and metaxylem areas, and trichome number and length was observed. Conditions were more unfavourable at the highest elevation that significantly reduced leaf with high proportion of parenchymatous region. Modifications such as increased proportion of storage parenchyma in midrib and lamina thicknesses are related to succulence (Fradera-Soler et al. 2021). Thicker epidermis and sclerenchymatous layer minimize water loss from leaf surface (Losada et al. 2021). Enhanced vascular bundle area and metaxylem area are interrelated with better solute conduction (Nja et al. 2018), while longer and dense trichomes shield leaves from desiccation and too much irradiance (Vassileva et al. 2019). All these relate to survival success in arid environments.

Population at 1000 m showed stem modifications such as increased epidermal and sclerenchymatous thickness, and areas of vascular bundle and metaxylem, which are modifications for xeric climate (Dörken et al. 2020). Leaf sheath thickness increased in this population, and the major contributor was cortical parenchyma. This will increase water storage capacity, and critical for drought-prone environments (Bano et al. 2019). Leaf anatomical traits in the population from 1000 m were most adversely affected showing a minimum of almost all traits except for stomatal density. Stomatal density was the maximum in this population that is associated with high transpiration rate (Bertolino et al. 2019). This will affect leaf growth and leaf anatomical traits (Tian et al. 2020).

Populations colonizing 800 m showed more developed stem and leaf sheath anatomical traits. Development of vascular and dermal tissues was the maximum in stem and leaf sheath, while intensity of sclerification increased in leaf sheath. All these traits are linked to xeromorphy by providing resistance to water movement outside the plant body, better conduction of solutes and increased mechanical support to parenchymatous tissue. Population

inhabiting 600 m showed the only modifications in stem, which were thick epidermis and broad metaxylem vessels, this contributes to increased xeromorphy (Honaine et al. 2016; Keshavarzi 2020).

5 Conclusion

Chrysopogon serrulatus showed a peculiar response at the highest elevation. Roots developed xeromorphy, where cells collapsed in the cortical region, metaxylem number and area significantly increased along with intensively sclerified stelar region. Stem cross-sectional area was greatly reduced at 1400 m elevation. Sclerification at stem periphery, especially outside vascular tissue increased, and overall response was the development of xeromorphic characters. The specific response of leaf sheath at the highest elevation was the development of large aerenchymatous cavities in the cortical region. Sclerification outside vascular tissue increased substantially in the leaf sheaths. Leaves of *C. serrulatus* developed succulence at 1400 m. Leaf width was greatly reduced but proportion of storage parenchyma significantly increased. Sclerification in leaves was reduced and stomatal shape was rhomboid. Eco-morphological and physio-anatomical features in *C, serrulatus* were influenced by habitat type, elevational range and other abiotic stresses. Such modifications guaranteed the survival of this species, and assisted to combat environmental stresses along elevational gradients, hence making it the most dominant component of vegetation in the Potohar region. Plants colonizing 1400 m are exposed to reduced growth period, extremely low temperatures, low availability of water and strong winds. At the highest elevation (1400 m), relative density, frequency, and cover, thus this species importance value, were observed to be dramatically less than even those values at 1200 m. In addition, plant size and dry weight were impressively less at 1200 and 1400 m than at the other 4 elevations. Many root, stem, leaf sheath and leaf blade morphological features demonstrated the greatest differences at the highest elevation. It was the highest elevation where temperatures were much milder, and precipitation was almost 4 times greater than that observed at the lowest elevation.

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