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ANATOMICAL MODIFICATIONS IN *CAREX BROWNII* AND *C. BRUNNEA* ALONG ELEVATION GRADIENT

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Abstract

The family Cyperaceae consists of more than 98 genera and 5500 species, primarily distributed in different habitats like wetlands and swamps, high elevations, hypersaline soils, deserts and semi-deserts, and water bodies. *Carex* is the most diverse genus of Cyperaceae that is restricted to cool temperate regions of northern hemisphere. The diversity and distribution of sedges change dramatically along elevation gradient with alterations in anatomical characteristics. The study was carried out to explore the anatomical modifications in naturally grown species of genus *Carex* along elevation gradient. Two species, *C. brownii*, and *C. brunnea*, were collected from three different elevations. All *Carex* species from various elevations showed variation. Soil physicochemical characteristics like ECe, TSS, SP, Cl^-, CO_3^{2-}, HCO_3^-, SO_4^{2-}, Na^+, Ca^{2+}, Mg^{2+}, K^+ in the habitats of *C. brunnea* showed strong association with morphological attributes at high elevation. Plant biomass was linked to high elevation in all three species. Anatomical traits like aerenchymatous cavities in *C. brunnea* increased significantly at the lowest elevation. In leaves, proportion of epidermis was the highest in *C. brunnea* at medium elevation. Vascular bundles were heavily sclerified in all species, maximally in *C. brunnea* at low elevation. High proportion of storage parenchymatous tissues was observed in roots of *C. brownii* and *C. brunnea* at medium elevation that help them to acclimatize water deficit environmental conditions by storing additional water. The *Carex* species showed unique adaptation along elevation gradient in their anatomical traits. Modifications at high elevation were high proportion of dermal tissue in root and leaves, stem vascular region and formation of root aerenchymatous cavities. Such modifications guarantee *Carex* spp. for successful survival in extremely cold environments at high elevations.

Key words: Biomass, Restoration, Storage parenchyma, Aerenchymatous cavities, Sclerenchyma

Abbreviations

Morphological traits: MPH–Plant height, MSL–Stem length, MRL–Root length, MIN–Inflorescence length, MLN–Number of leaves per plant, MLA–Leaf area, MFW–Plant fresh weight, MDW–Plant dry weight.
Soil physicochemical and environmental attributes: ETX–Maximum annual temperature, ETN–Minimum annual temperature, ERF–Rainfall, ESF–Snowfall, EWS–Windspeed, EHM–Relative humidity, OPH–Soil pH, OEC–Soil ECe, OTS–Soil Total soluble salts, OSP–Saturation percentage, OOM–Organic matter, OCL–Soil Cl^-, OHC–Soil HCO_3^-, OHS–Soil SO_4^{2-}, ONA–Soil Na+, OCA–Soil Ca^{2+}, OMG–Soil Mg^{2+}, OK+–Soil K^+, OPS–Olsen phosphorus.
Root anatomical traits: RRD–Root radius, RET–Epidermal thickness, ROC –Outer cortical region thickness, ROA–Outer cortical cell area, RIC–Inner cortical region thickness, RIA–Inner cortical cell area, REN–Endodermis thickness, RMX–Metaxylem area, RST–Stelar region thickness, RAE–Aerenchymatous area.
Stem anatomical traits: SRD–Stem radius, SET–Epidermal thickness, SCT–Cortical region thickness, SCA–Cortical cell area, SVB–Vascular bundle area, SMX–Metaxylem area, SPH–Phloem area, SAE–Aerenchymatous area.
Leaf anatomical traits: LMT–Midrib thickness, LLT–Lamina thickness, LMS–Mesophyll thickness, LDE –Adaxial epidermal thickness, LBE–Abaxial epidermal thickness, LVB–Vascular bundle area, LMX –Metaxylem area, LPH–Phloem area, LAE –Aerenchyma area, LBF –Bulliform thickness, LDA–Adaxial epidermal cell area, LBA–Abaxial epidermal cell area, LSA–Abaxial stomatal area, LSB–Abaxial stomatal density.

Introduction

Global climate change has a great impact on biodiversity of freshwater wetlands (Moomaw *et al*., 2018) which significantly affects species diversity, vegetation structure and the number of invasive species (Albert *et al*., 2021). Ecological factors like temperature and soil nutrients are important for community structure and distribution of species, especially the sedges (Fan *et al*., 2019). The genus *Carex* is widely distributed in cool temperate areas of the world. Rise in temperature is responsible for upward shift of plant species along elevation gradient.

The genus *Carex* includes high elevation species that primarily spread in northern and moist areas of Pakistan (Metrak *et al*., 2015). Species of *Carex* genus, *C. brownii* and *C. brunnea* were collected from different elevations of Donga Galli Punjab (2300 m), Thandiani Khyber Pakhtoonkhwa (2800 m) and Banjosa Lake Azad Jammu and Kashmir (1800 m). *Carex brownii* Tuckerman is common sedge occurring in the Himalayan region of Pakistan (Haq *et al*., 2011). *Carex brownii* have perennial growth habit, short rhizome, hollow triangular stem and roots arranged in clustered manner near the base of stem. *Carex brunnea* Thunb. is commonly known as greater brown sedge of northern, temperate and tropical regions of Asian and Africa countries throughout the world. This species is frequently distributed in the Himalayan region up to 2800 m elevation (Kukkonen, 2001).

Plants adapted to high elevation are structurally different from those colonize lower elevations (Ahmad *et al*., 2020). Environmental stresses are undeniably responsible to cause variations in plant behavior along the altitudinal gradient (Guerin *et al*., 2012). High altitude plants usually have thick and rigid cell walls, thick mesophyll, higher stomatal density, and dense pubescence (Shi *et al*., 2015; Ahmad *et al*., 2016a). Plants growing at high elevations have reduced epidermal cell area and increased sclerification around the vascular tissues (Ahmad *et al*., 2016b). Plants present at high elevation have thick and rigid cell wall that help them to acclimatize low temperature and escape cellular damages (Shi *et al*., 2015).

At high elevations, chilling temperatures between zero and 10°C reduce leaf functions by inhibiting the xylem route (Ahmad *et al*., 2016b). Wind speed and snow cover on the plant, cause abrasive damage to the leaf cuticle and reduce stomatal conductance. In high-altitude plants species, the thickness of the leaf midrib increases with the rise in elevation (Ahmad *et al*., 2016a). Leaf thickness is increased due to increased deposition in mesophyll cells of leaf, high concentration of CO_2, increased stomatal number and large number of trichomes (Ahmad *et al*., 2016b).

Herein, it is hypothesized that changes in structural and functional features may assist *Carex* species for the survival in freezing cool temperatures. On these bases, the research questions to be addressed were a) how morpho-anatomical and soil-physicochemical traits contribute in the survival of *Carex* species at different altitudes, and b) why *Carex* species are restricted to freezing temperatures. The present study was focused on the evaluation of morpho-anatomical adaptations in two *Carex* species, *C. brownii* and *C. brunnea* at different elevations.

Materials and Methods

Genus *Carex* (family Cyperaceae) is the one of the most diverse genus of the flowering plants. Two *Carex* species (*C. brownii* and *C. brunnea*) were collected from Banjosa Lake-low (1800 m), Donga Galli-medium (2300 m) and Thandiani-high (2800 m a.s.l.) elevation in the Punjab, Khyber Pakhtoonkhwa and Azad Jammu and Kashmir during 2018-20 (Fig. 1). Six fully mature plants (replication) at flowering stage of each species were collected randomly from each collection site during October 2019. Environmental attributes of the selected sites are presented in (Table 1).

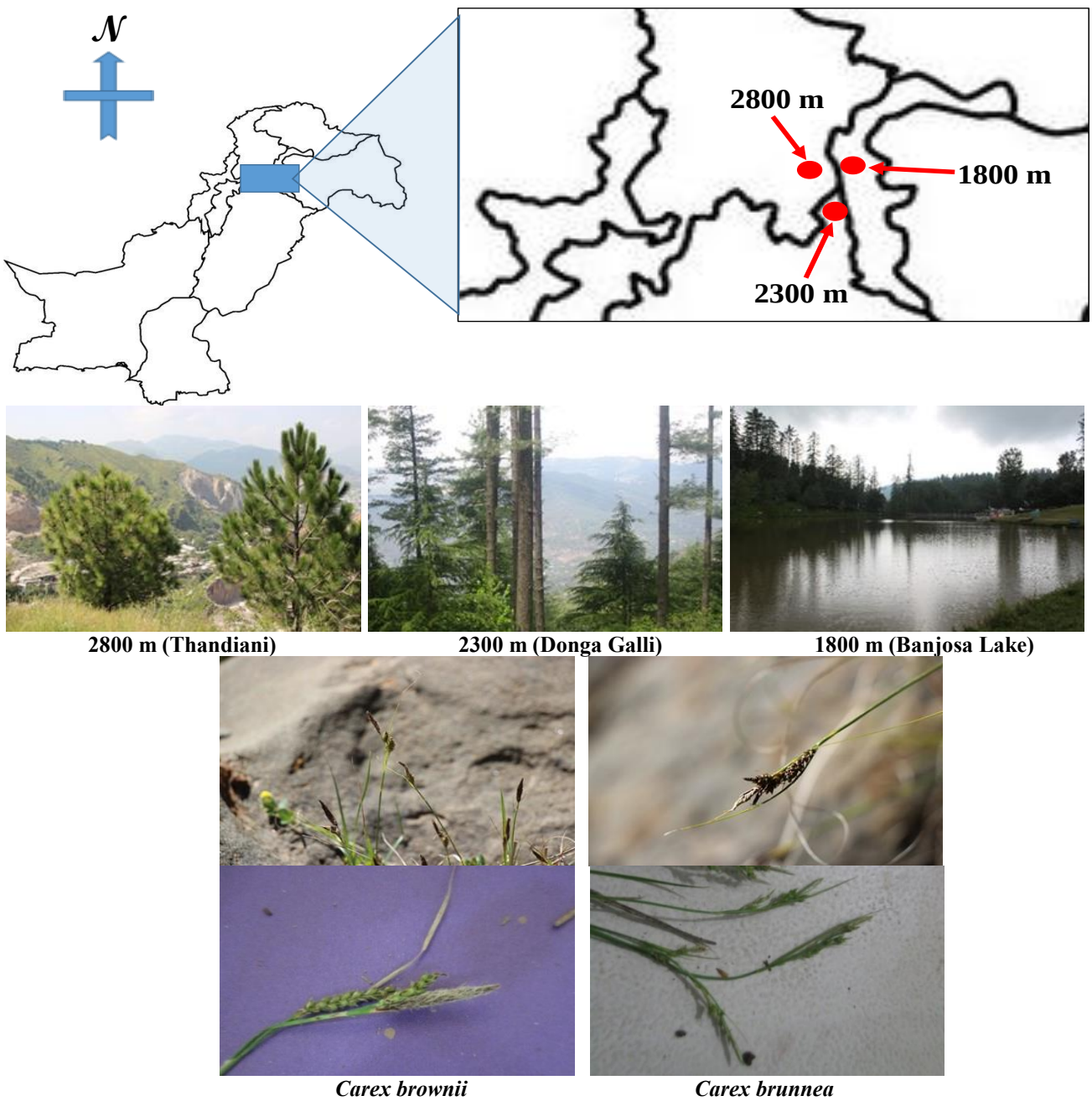


Fig. 1. Map showing collection sites of two *Carex* species at different elevations in Punjab, Khyber Pakhtoonkhwa and Azad Jammu and Kashmir.

Table 1. Environmental attributes of the collection sites of *Carex* species.

Collection site	High	Medium	Low
District	Abbottabad	Rawalpindi	Poonch
Elevation (m a.s.l.)	2800	2300	1800
Longitude	73°18'51" N	73°23'36" N	73°49'04" N
Latitude	34°11'25" E	34°03'34" E	33°48'35" E
Slope (%)	70	65	55
Aspect	Northnorthwestern	Northwestern	Northeastern
Dominant species	*Pwa, Cde, Acy*	*Pro, Tan, Maf*	*Pwa, Pro, Sat*
Maximum annual temperature (°C)	21	25	28
Absolute maximum temperature (°C)	32	34	33
Minimum annual temperature (°C)	1	2	2
Absolute Minimum temperature	–4.9	–1.6	–0.5
Rainfall (mm)	1603	1597	1210
Snowfall (mm)	15.1	16.2	10.5
Wind speed (Km h^{-1})	15	13	12
Relative humidity (%)	34	33	32

Pwa-Pinus wallichiana, Cde-Cedrus deodara, Acy-Aristida cyanantha, Pro-Pinus roxburghii, Tan-Themeda anathera, Maf-Myrsine africana, Sat-Strobilanthes atropurpurea

Soil physicochemical traits: Soil from the root rhizosphere was taken at the depth of 15-20cm to analyze physicochemical characteristics. Soil (500 g) was taken, completely air-dried for the preparation of homogenous paste for estimating pH, ECe, ionic content and moisture contents. Soil pH was determined by pH meter (Hanna H8417 USA), while ECe by electrical conductivity meter (WTW Conductometer LF 191, Germany). Total soluble salts (TSS) were determined by following Handbook 60 Richards (1954). Saturation percentage was measured by given formula:

$$\text{Saturation percentage} = \frac{\text{Weight of saturated paste}}{\text{Oven dried saturated paste}} \times 100$$

Soil organic matter (OM) was determined by titrating 1g soil sample against 0.5N ferrous sulphate solution by using ferroin indicator to get light green end point. Carbonates from the soil extract were determined by using phenolphthalein as indicator to a colorless endpoint. Bicarbonates from the soil sample were determined by the same extract that used for the determination of carbonates to a golden yellow end point by using methyl orange as an indicator. Chloride from the soil extract was titrated against N/100 silver nitrate solution to a brick red end point using a potassium chromate as indicator. Sulphate from the soil was determined by difference method (nitrate was not included in the formula).

TSS – Chloride – Carbonates – Bicarbonates = Sulphate

Soluble calcium magnesium (CaMg) was determined from the saturated soil extract by using eriochrome black tea as indicator to a bluish green end point by titrating it against N/100 (EDTA) ethylene diamine tetra acetic acid solution. Soluble Na^{+}, K^{+} and Ca^{2+} were recorded by using flame photometer (Jenway, PFP-7, USA). Soluble magnesium content from the soil extract was determined by deducting the calcium from the soluble extract of CaMg. Phosphorus from the soil was determined by extraction with 0.5 M sodium bicarbonate solution and shaking for 30 minutes on reciprocating shaker subsequently 5ml was taken in 50ml conical flask then added 5 ml color developing reagent in the conical flask and made the volume up to the 50ml. Phosphorus was determined on the spectrophotometer (APEL spectrophotometer PD-303S) at 880 nm wavelength (Olsen and Watanabe, 1964).Soil texture was determined by hydrometer method (Moodie *et al*., 1959) Soil physicochemical characteristics of the collection sites are given in (Table 2).

Morphological parameters: Plants samples were randomly collected keeping a distance of 3 m among individual plants. Fresh weights were measured directly on a portable electric balance (Model: FA2004B, YK Scientific Instrument China). These samples were stored and transported back to the laboratory to record data for other morphological features. Collected plants samples were oven-dried at 60°C for one week to get a constant weight. Electrical balance was used to measure the dry weights. Plant height was taken from base to top of the stem. Inflorescence length and stem length was measured by meter rule. Leaf area of every leaf was calculated by the formula Lopes *et al*., (2016).

$$\text{Leaf area } (cm^2) = \frac{\text{Maximum length x Maximum width x 0.68 (correction factor)}}{\text{Number of leaves on each plnat were counted}}$$

Anatomical parameters: To determine anatomical characteristics, a small portion (2-cm) of stem (topmost internode, root (root-shoot junction), leaf (basal portion of the largest leaf) and leaf sheath (basal portion of the same leaf) were collected. The material was preserved in formalin acetic alcohol (FAA) solution containing 5% formalin, 50% ethyl alcohol, 10% acetic acid, and 35% distilled water. Afterwards, plant material was shifted to acetic alcohol 75% ethyl alcohol and 25% acetic acid for long time storage (Ahmad *et al*., 2018).

Table 2. Soil physicochemical parameters of the collection sites of *Carex brownii* and *C. brunnea* collected from different elevations.

Traits	Elevation (m a.s.l.)	*Cbw*	*Cbu*
pH	1800	7.12	7.09
	2300	7.2	7.05
	2800	7.34	7.08
ECe (dS m^{-1})	1800	1.22	0.65
	2300	0.93	0.43
	2800	1.55	1.73
Saturation percentage (%)	1800	33.36	38.41
	2300	37.52	25.28
	2800	36.11	48.11
Organic matter (%)	1800	0.58	0.69
	2300	0.64	0.78
	2800	0.46	0.64
Total soluble salts (mg Kg^{-1})	1800	780.8	1107.2
	2300	595.2	275.21
	2800	992.0	416.01
Soil texture	1800	Loam	Loam
	2300	Loam	Sandy loam
	2800	Loam	Loam
Soil K^+ (mg Kg^{-1})	1800	7.69	5.7
	2300	6.01	1.48
	2800	8.32	10.53
Soil Na^+ (mg Kg^{-1})	1800	11.34	9.62
	2300	12.76	3.31
	2800	13.35	17.79
Soil Ca^{2+} (mg Kg^{-1})	1800	54.52	31.38
	2300	45.09	14.58
	2800	77.53	117.17
Soil Mg^{2+} (mg Kg^{-1})	1800	8.26	4.76
	2300	7.06	2.21
	2800	11.75	17.76
Olsen phosphorus (mg Kg^{-1})	1800	8	8.60
	2300	7.84	9.20
	2800	7.36	7.60
Cl^- (mg Kg^{-1})	1800	7.09	11.57
	2300	10.64	5.64
	2800	8.96	37.86
Soil HCO_3^- (mg Kg^{-1})	1800	162.8	93.73
	2300	183.12	40.71
	2800	88.12	117.40
Soil SO_4^{2-} (mg Kg^{-1})	1800	57.68	31.54
	2300	9.01	13.35
	2800	187.31	256.72

Cbw–Carex brownii, Cbu–Carex brunnea

Plant sections were cut by free-hand sectioning method by using double edge razor blade. The plant section was dehydrated with ethyl alcohol to prepare permanent slides (Ruzin, 1999). Sclerenchyma tissues were stained by safranin, while living parenchyma tissues by fast green. Transverse sections were fixed on a glass slide by adding a drop of DPX and then covered it by a coverslip. Photographs of permanent slides were taken by camera-equipped digital compound microscope (MT4300-LV-HD, Meiji Techno, Japan) All readings related to tissues were recorded with the help of ocular micrometer, which was calibrated by stage micrometer.

Percentages of each tissue proportion were calculated, and then summed up the percentages of following growth parameters.

Morphology: Biomass (plant fresh and dry weights), leaves (number of leaves per plant and leaf area), length (plant height, inflorescence length, root length and stem length).

Root anatomy: Vascular (metaxylem area), Parenchymatous (outer cortical region thickness outer cortical cell area, inner cortical region thickness, inner cortical cell area and stellar region thickness), Dermal (epidermal and endodermal thicknesses) and Aerenchymatous (aerenchyma area).

Stem anatomy: Aerenchyma (aerenchyma area), Vascular (vascular bundle area, metaxylem area and phloem area), Parenchymatous (cortical region thickness and cortical cell area), Dermal (epidermal thickness) and Radius (stem radius).

Leaf anatomy: Stomata (adaxial stomatal area and adaxial stomatal density), Vascular (vascular bundle area, metaxylem area and phloem area), Parenchymatous (aerenchymatous area and mesophyll thickness), Thickness (midrib thickness, lamina thickness), Dermal (adaxial epidermal thickness, abaxial epidermal thickness and bulliform thickness).

Results

Soil physicochemical characteristics: Soil of *C. brownii* revealed the maximum pH (7.34) at 2800 m. *Carex brownii* and *C. brunnea* showed the maximum ECe (1.73 dS m^{-1}), soil Ca^{2+} (117.17 mg kg^{-1}) and soil Mg^{2+} (17.76 mg kg^{-1}) at 2800 m (Table 2). Saturation percentage of soil K^{+}, soil Na^{+} and soil Cl^{-}were the maximum in *C. brunnea* at 2800 m. Organic matter was the maximum (0.78%) in *C. brunnea* at 2300 m. Total soluble salts was the maximum (1107.2 mg kg^{-1}) in *C. brunnea* at 1800 m, while in *C. brownii* at 2800 m. Soil texture was loamy in *C. brownii* at all elevations, whereas that of *C. brunnea* sandy loamy at 2300 m. Olsen phosphorus was the maximum (9.20 mg kg^{-1}) in *C. brunnea* 2300 m. Soil HCO_3^{-} was the maximum (183.12 mg kg^{-1}) in *C. brownii* at 2300 m, while in *C. brunnea* at 2800 m. Soil SO_4^{2-} was the maximum in *C. brownii* (187.31 mg kg^{-1}) and *C. brunnea* (256.72 mg kg^{-1}) at 2800 m.

Morphology: In *C. brownii,* proportion of leaves and length increased significantly, but plant biomass decreased at 1800 m. Biomass production in *C brownii* increased at 2300 m, while proportion of length was the maximum at 2800m. In *C. brunnea,* proportion of length and biomass was the highest at 2800 m, whereas leaves were the maximum in *C. brownii* and *C. brunnea* at 1800 m (Fig. 2).

Root anatomy: Root anatomical traits in *C. brownii* generally not responded to elevation gradient; only proportion of dermal tissue increased significantly at 2800 m. Aerenchymatous development was the maximum in *C. brunnea* at 1800 m. Size of aerenchymatous cavities reduced significantly in *C. brunnea* at 2300 m, whereas proportion of dermal and parenchymatous tissues were the minimum in this species at 2800 m. Parenchymatous proportion was maximum in *C. brownii* and *C. brunnea* at 2300 m (Figs. 2 and 3).

Stem anatomy: Proportion of aerenchymatous tissues was the maximum in *C. brownii* at 1800m, whereas proportion of aerenchymatous tissues and radius were significantly reduced in this species at 2300m. Aerenchymatous formation was the maximum in *C. brunnea* at 1800m and 2800m, whereas that of dermal and vascular tissues was the minimum at 2800m. Parenchymatous tissues was the maximum in *C. brunnea* at 2300 and 2800m (Figs. 2 and 3).

Leaf anatomy: Proportion of vascular, parenchymatous, dermal tissues and thickness in *C. brownii* was greatly decreased at 2300 m. The proportion of vascular and parenchymatous tissues was significantly increased in this species at 1800 m. In *C. brunnea,* proportion of vascular, parenchymatous thickness was significantly increased at 2300m, whereas these anatomical traits were significantly reduced in this species at 2800 m. (Figs. 2, 4 and 5).

PCA analysis

Morphological, soil and environmental traits: In *C. brownii,* morphological attributes root length showed weak association with soil organic matter and HCO_3^{-} at 2300 m (Fig. 6). Plant height and leaf area revealed close association with soil K^{+}, Mg^{2+}, Ca^{2+}, SO_4^{2-}, ECe and total soluble salts at 2800 m. Soil phosphorus showed no association with minimum annual temperature at 1800 m. In *Carex brunnea,* the number of leaves per plants showed close relationship with annual minimum temperature at 1800 m. Soil organic matter revealed close relationship with Olsen phosphorus at 2300 m. Morphological attributes like stem length, fresh weight and number of leaves per plants showed close association with soil Cl^{-}, Ca^{2+}, Mg^{2+}, SO_4^{2-}, ECe, total soluble salts, wind speed, maximum annual temperature and relative humidity at 2800m.

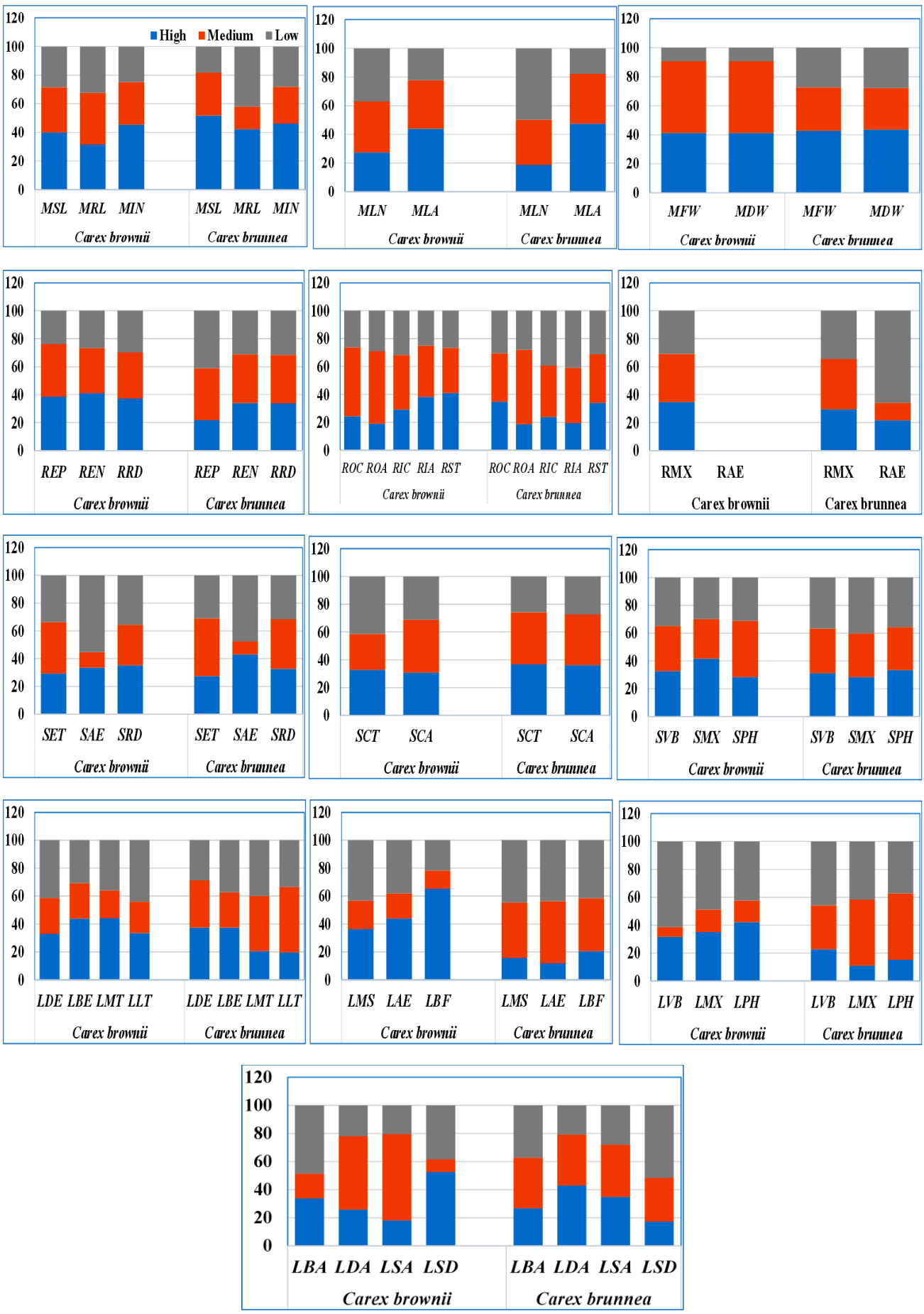


Fig. 2. Morpho-anatomical traits of two *Carex* species collected from three different elevations.

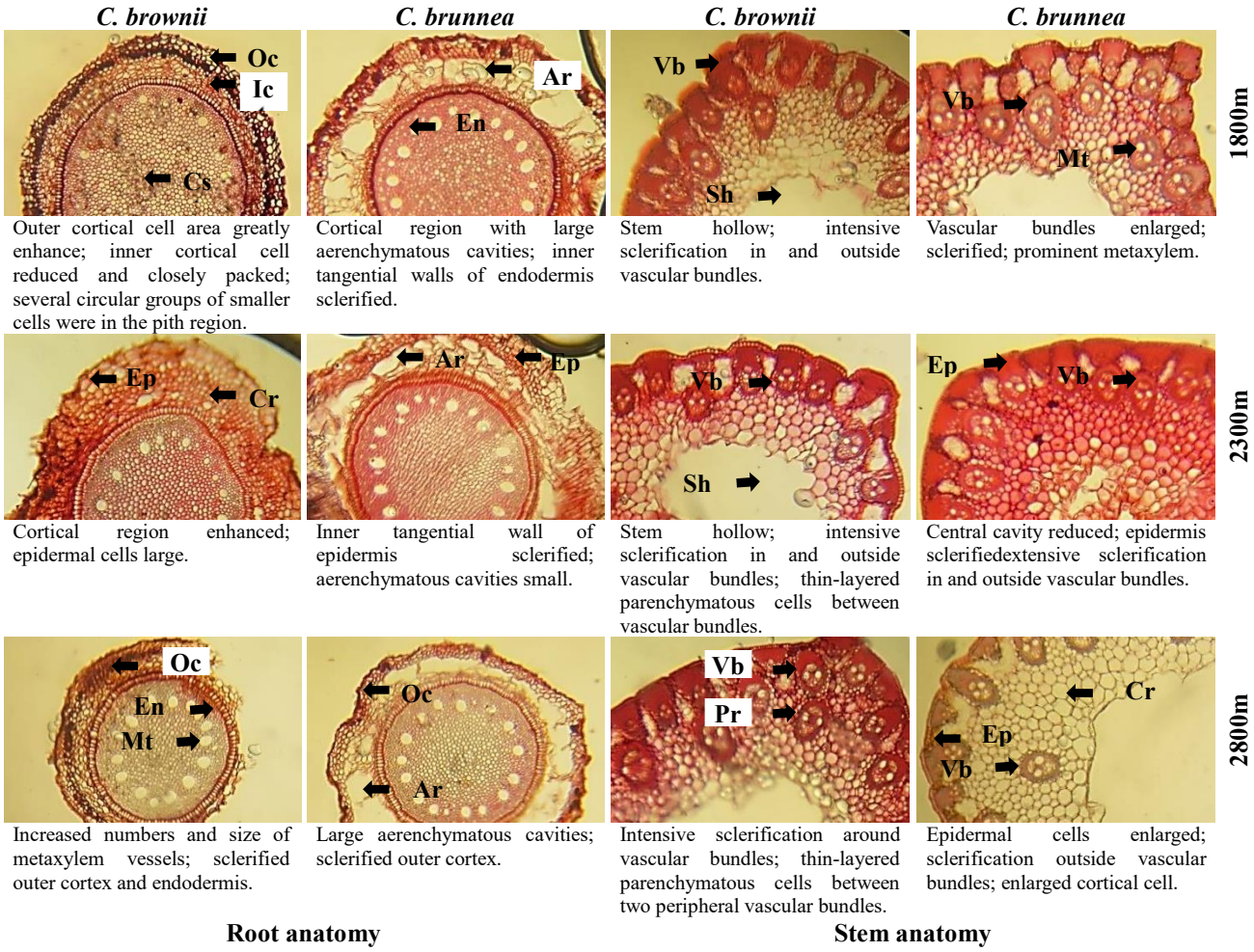


Fig. 3. Root and stem transverse section of two *Carex* species collected from different elevations in the Punjab, Khyber Pakhtoonkhwa and Azad Jammu and Kashmir.
Oc-Outer cortical cell area, Ic- Inner cortical cell area, Cc-Cortical cells, Cs-Circular smaller cells, Ar-Aerenchymatous cavities, En-Endodermis, Ep-Epidermis, Sr-Stelar region, Cr -Cortical region, Mt-Metaxylem vessels, Sh-Stem hollow, Vb-Vascular bundle, Mt-Metaxylem area, Cr-Cortical cell, Ar-Aerenchymatous cavities, Ep-Epidermis, Pr-Parenchymatous cells.

Root and stem anatomical, soil and environmental traits: In *C. brownii,* Olsen phosphorus showed close association with root anatomical attributes like stelar region thickness and aerenchymatous area. The minimum annual temperature was strongly associated with stem anatomical attributes like vascular bundle area, cortical region thickness and aerenchymatous area at 1800 m (Fig. 7). Among stem anatomical traits, epidermal thickness, cortical cell area, phloem area and among root anatomical attributes outer cortical region thickness, outer cortical cell area and inner cortical region thickness were linked to soil organic matter and HCO_3^- at 2300 m. Stem radius and metaxylem area were strongly associated with soil Ca^{2+}, SO_4^{2-}, Mg^{2+}, ECe, total soluble salts and K^+ at 2800 m.

In *C. brunnea,* root aerenchymatous area and stem anatomical attributes like vascular bundle area, metaxylem area and phloem area showed close relationship with minimum annual temperature at 1800 m (Fig. 7). Soil organic matter showed close association with root outer cortical cell area, stem radius and epidermal thickness, whereas Olsen phosphorus was associated with root metaxylem area and inner cortical cell area at 2300 m. Soil pH, showed close association with stem aerenchymatous area, while soil physicochemical traits like K^+, HCO_3^-, saturation percentage, Na+, Mg^{2+}, Cl^-, SO_4^{2-}, ECe, Ca^{2+}, and total soluble salts were clustered in a same group at 2800 m.

Leaf anatomical, soil and environmental traits: The minimum annual temperature in *C. brownii* was strongly associated with adaxial epidermal thickness, abaxial epidermal cell area, lamina thickness, metaxylem area, mesophyll thickness, vascular bundle area, phloem area, and aerenchyma area at 1800 m (Fig. 7). Soil ECe, K^+, HCO_3^-, Mg^{2+}, Ca^{2+} and total soluble salts revealed strong association with leaf anatomical traits like bulliform thickness, abaxial epidermal thickness, abaxial stomatal density and midrib thickness at 2800 m. Among leaf anatomical parameters, adaxial epidermal cell area and abaxial stomatal area clustered together at 2300 m.

In *C. brunnea,* minimum annual temperature showed a close association with leaf anatomical attributes like vascular bundle area and abaxial stomatal density at 1800 m (Fig. 7). Soil organic matter and Olsen phosphorus were closely associated with lamina thickness, phloem area, metaxylem area and aerenchyma area at 2300m. Environmental attributes like maximum annual temperature, wind speed, relative humidity, rainfall, and snow fall showed a relationship with adaxial epidermal cell area, adaxial epidermal thickness and abaxial stomatal area at 2800m.

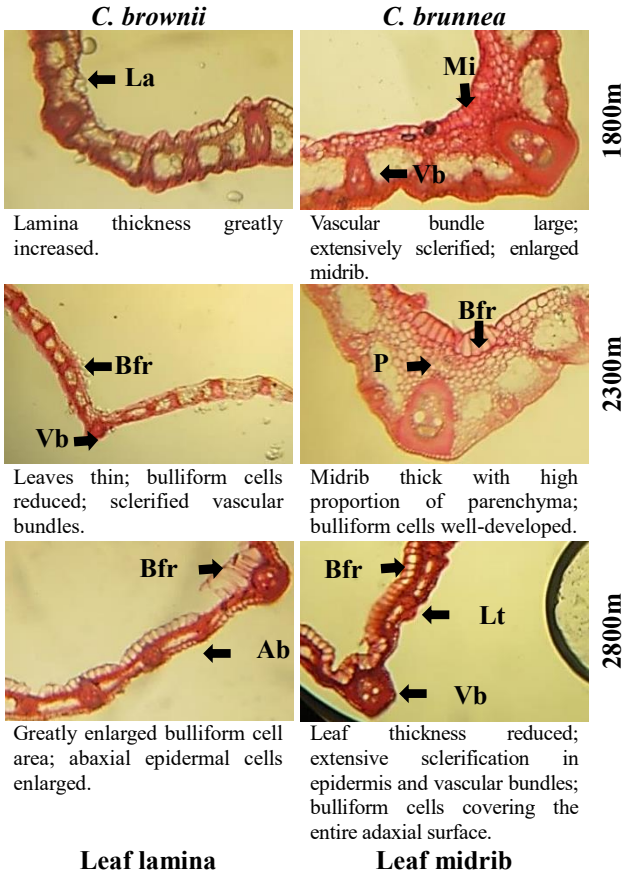


Fig. 4. Leaf transverse section of two *Carex* species collected from different elevations in the Punjab, Khyber Pakhtoonkhwa and Azad Jammu and Kashmir
La-Lamina, Mi-Midrib, Vb-Vascular bundle, Ar-Aerenchymat cavities, Bf-bulliform cell, P-Parenchyma, Ab-Abaxial epidermal cell, Lt-Leaf thickness

Discussion

Species composition and distributional pattern alter along elevation gradient. Climate change can significantly change species associations and hence resulting in uplift on higher elevations of more sensitive species (Pacifici *et al*., 2015). A significant relationship exists between species number and distributional pattern. Many strategies and mechanisms are adopted by plant species to survive under freezing temperatures. Variation in structural and functional features in *Carex* species was recorded from Thandiani (high elevation), Donga Galli (medium elevation) and Banjosa Lake (low elevation). Plasticity in leaf structural and functional features helps the plant to adjust with changing environment (Nicotra *et al*., 2010). Structural and functional responses are critical to understand the behavior of plants with changing climate. Wide distributional range is directly related to species heterogeneity and structural and functional plasticity (Salama *et al*., 2018).

Among length related traits, stem length and inflorescence length were the maximum at high elevation in *C. brownii* and *C. brunnea*. Temperature in winters reach below 0°C at high elevation, the area received heavier snowfall than the medium and low elevations. Cold tolerant species generally had structural and functional plasticity and showed better growth at low temperatures because of better adaptability of low temperature acclimation (Yamori *et al*., 2010). Chambers *et al*., (2017) reported an increase in root length, which increased by 50% in herbaceous plants at high elevations. Root length increases only in *C. brunnea* at the highest elevation in our studies, indicating more cold tolerance of this species.

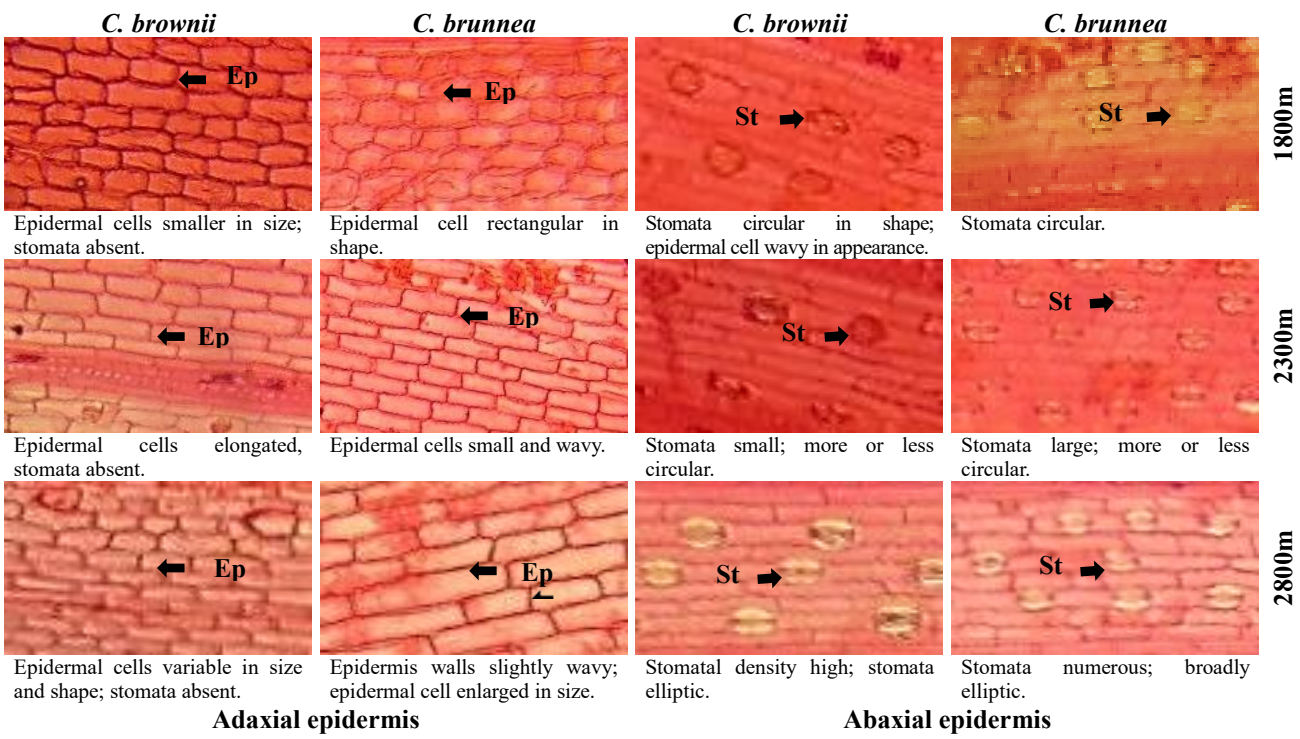
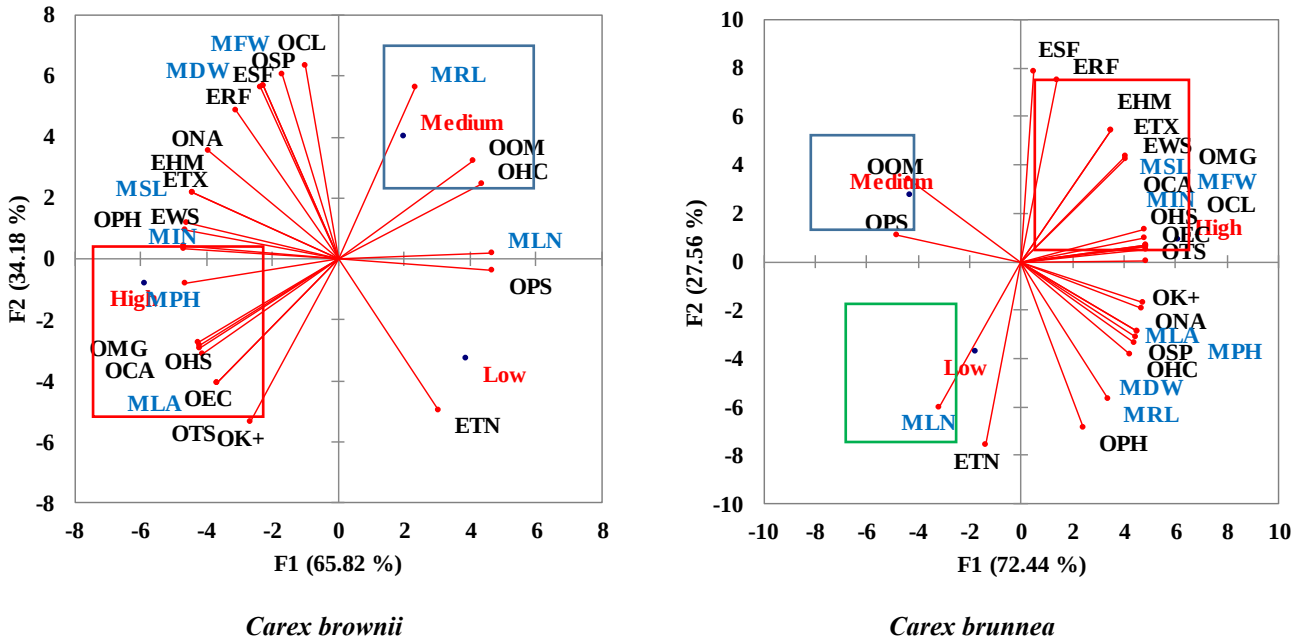


Fig. 5. Epidermal peel of adaxial and abaxial leaf surface of two *Carex* species collected from different elevations in the Punjab, Khyber Pakhtoonkhwa and Azad Jammu and Kashmir
Ep-Epidermal cell. St-Stomata



Carex brownii *Carex brunnea*

Fig. 6. Relationship among environmental, soil physicochemical and morphological traits of two *Carex* species along elevation gradient. **Environmental:** Elevations: Low (1800m), medium (2300m), high (2800m). ETX–Maximum annual temperature, ETN–Minimum annual temperature, ERF–Rainfall, ESF–Snowfall, EWS–Windspeed, EHM–Relative humidity **Soil physicochemical:** OPH–Soil pH, OEC–Soil ECe, OTS–Soil Total soluble salts, OSP–Saturation percentage, OOM–Organic matter, OCL–Soil Cl^-, OHC–Soil HCO_3^-, OHS–Soil SO_4^{2-}, ONA–Soil Na+, OCA–Soil Ca^{2+}, OMG–Soil Mg^{2+}, OK+–Soil K^+, OPS–Olsen phosphorus. **Morphology:** MPH–Plant height, MSL–Stem length, MRL–Root length, MIN–Inflorescence length, MLN–Number of leaves per plant, MLA–Leaf area, MFW–Plant fresh weight, MDW–Plant dry weight.

Morphological parameters like inflorescence length and leaf area are influenced along altitudinal gradient in cold tolerant polymorphic species like *Dracocephalum nutans* with broad distributional range from low mountains to alpine belt. In the present study, inflorescence length and leaf area increased at high elevation, while number of leaves were the maximum at low elevation. This indicates the better adaptability potential of both *Carex* species to freezing temperature at high elevations. Read *et al*., (2014) observed a decrease in number of leaves per plant at high elevations, whereas Zhang *et al*., (2012) reported a decreased leaf area in forest communities. A reduction in leaf area at high elevation in less tolerant species protects leaf membranes, and hence plants from wilting (Jiang *et al*., 2011).

Plant biomass production provides a suitable way to understand the effect of climate on plant growth and development (Hatfield *et al*., 2015), and also to study the survival mechanism against environmental stresses (Antar *et al*., 2021). A decreased plant biomass percentage (plant dry weight and fresh weight) was recorded in *C. brownii* from high elevation. Similar findings were reported by Fatima *et al*., (2018) in *Aristida adscensionis* at high altitude, this is because of unfavorable environment and reduced growth period for growth and development. Biomass (plant fresh and dry weights) increased at medium elevation in *C. brownii,* while at high elevation in *C. brunnea,* indicating better adaptation of the latter species to cold temperatures.

Anatomical attributes are more sensitive to environmental adversaries as compared to morphological and physiological attributes (Mansoor *et al*., 2019). Root dermal tissue (mainly epidermal thickness) provides protection to metabolically active parenchymatous tissues from extremely cool environmental condition that guaranteed their survival (Karabourniotis *et al*., 2021). Dermal tissue increased in *C. brownii* colonizing the highest elevation, whereas unaltered in *C. brunnea. Carex brownii* may require more protection to survive in cold temperatures than more adapted *C. brunnea,* because increased epidermis and endodermis provide environmental barrier critical for survival. Ahmad *et al*. (2016) reported a continuous decrease in root epidermal cell area and cortical region thickness in less tolerant *Koeleria macrantha* with the rise in elevation.

The proportion of vascular tissue significantly reduced in *C. brunnea* at the highest elevation. Graefe *et al*., (2011) observed a decrease in vessel diameter at high elevations that affected the water uptake by roots. Reduction in vascular tissue proportion (metaxylem area and vascular bundle area) along elevation gradient is linked to the survival in extremely cold environment, as it reduces the chance of cavitation in xylem vessel (Fatima *et al*., 2022).

The increased proportion of aerenchymatous cavities was recorded in roots of *C. brunnea* at low elevation. Aerenchymatous cavities promote gaseous exchange inside and outside the plant body (Hoffmann *et al*., 2019) and facilitate the movement of organic solutes, water and essential nutrients (Sorrell *et al*., 2013). Karlova *et al*., (2021) reported aerenchyma formation in roots under abiotic stresses including cold stress.

Environmental gradient has direct impact on the stem anatomical attributes along the rise in elevation. Stem anatomical attributes showed reduction in parenchymatous, vascular, dermal tissues proportion in *C. brownii* and *C. brunnea* at high elevation. Ahmad *et al*., (2016) reported reduced proportion of vascular bundle area and metaxylem area in Aveneae grasses colonizing cold climates of high elevation in the Himalayan region (Ahmad *et al*., 2016). In plants from cool arid habitats, vascular tissue is contracted and randomly distributed around small parenchymatous tissue (Rakic *et al*., 2017). High proportion of parenchymatous tissues was recorded in *C. brunnea* at the highest elevation. In contradictory, enlarged cortical parenchymatous cells were observed at

the highest elevation, which is connected with enhanced storage capacity (Kulkarni & Deshpande, 2006), and is extremely beneficial in water scarce condition in the cool mountainous regions (Fatima *et al*., 2022).

The proportion of stomata (stomatal area and density) was the highest in *C. brownii* and *C. brunnea* from low elevations, which decreased at the highest elevations. This modification is a major adaptive feature to survive under adverse environmental conditions by regulating transpiration rate (Iqbal *et al*., 2020). Stomatal density and stomatal area are associated with water use efficiency that directly linked to low transpiration rate (Merced *et al*., 2017). Stomata were recorded only on the abaxial leaf surface. The adaxial surface is more exposed to climatic adversaries like extremely cold temperatures, strong winds and irradiance. Stomata observed only on the abaxial leaf surface, hence stomatal orientation in this case is significantly important for a plant inhabiting high elevations by reducing transpiration rate ensuring water conservation (Guo *et al*., 2022).

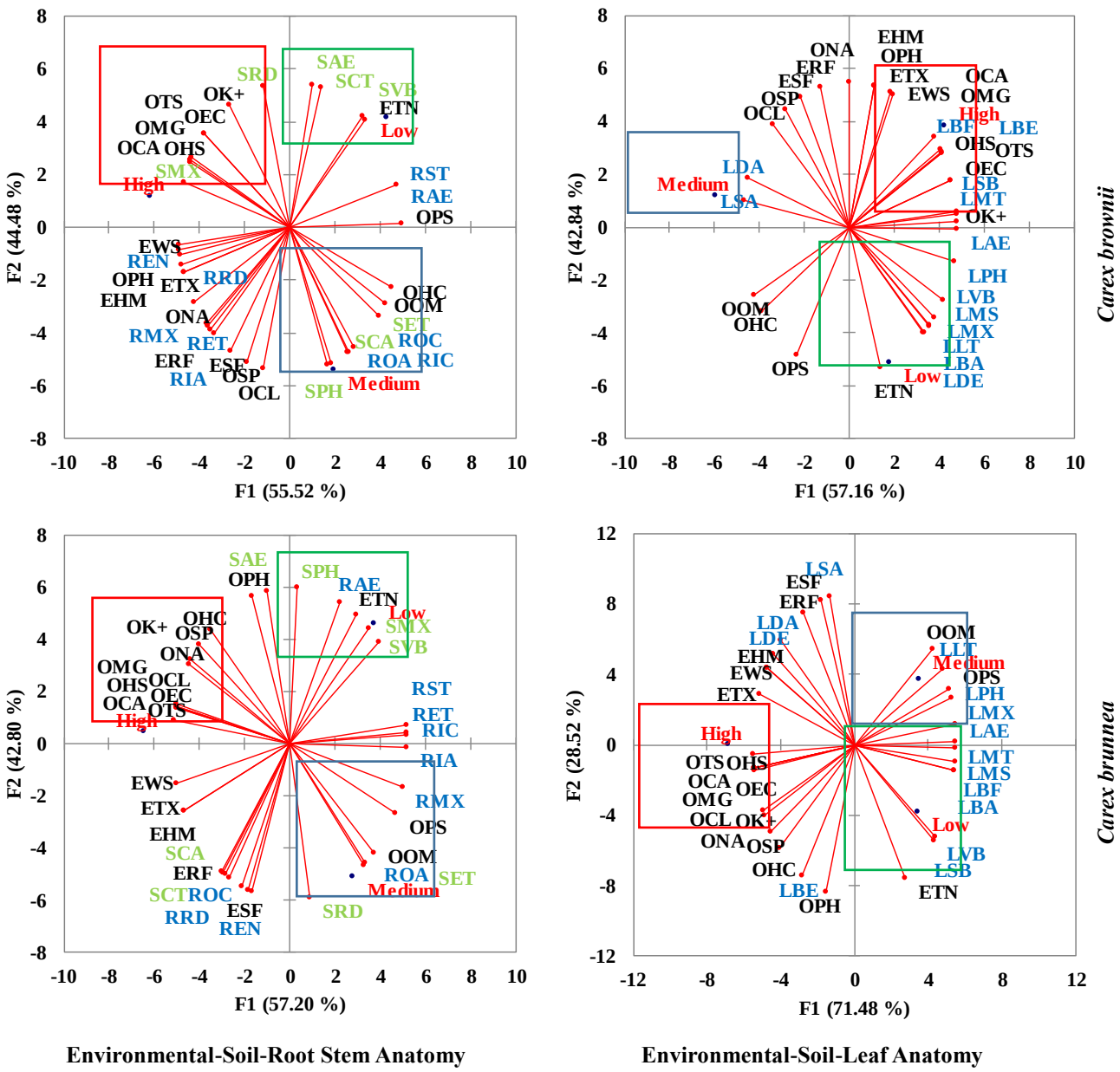


Fig. 7. Relationship among environmental, soil physicochemical and anatomical traits of two *Carex* species along elevation gradient.
Environmental: Elevations: Low (1800m), medium (2300m), high (2800m). ETX–Maximum annual temperature, ETN–Minimum annual temperature, ERF–Rainfall, ESF–Snowfall, EWS–Windspeed, EHM–Relative humidity **Soil physicochemical:** OPH–Soil pH, OEC–Soil ECe, OTS–Soil Total soluble salts, OSP–Saturation percentage, OOM–Organic matter, OCL–Soil Cl^-, OHC–Soil HCO_3^-, OHS–Soil SO_4^{2-}, ONA–Soil Na+, OCA–Soil Ca^{2+}, OMG–Soil Mg^{2+}, OK+–Soil K^+, OPS–Olsen phosphorus., **Root anatomy:** RRD–Root radius, RET–Epidermal thickness, ROC –Outer cortical region thickness, ROA–Outer cortical cell area, RIC–Inner cortical region thickness, RIA–Inner cortical cell area, REN–Endodermis thickness, RMX–Metaxylem area, RST–Stelar region thickness, RAE–Aerenchymatous area, **Stem anatomy:** SRD–Stem radius, SET–Epidermal thickness, SCT–Cortical region thickness, SCA–Cortical cell area, SVB–Vascular bundle area, SMX–Metaxylem area, SPH–Phloem area, SAE–Aerenchymatous area **Leaf anatomy:** LMT–Midrib thickness, LLT–Lamina thickness, LMS–Mesophyll thickness, LDE –Adaxial epidermal thickness, LBE–Abaxial epidermal thickness, LVB–Vascular bundle area, LMX –Metaxylem area, LPH–Phloem area, LAE –Aerenchyma area, LBF –Bulliform thickness, LDA–Adaxial epidermal cell area, LBA–Abaxial epidermal cell area, , LSA–Abaxial stomatal area, LSB–Abaxial stomatal density.

The proportion of vascular tissue (vascular bundle area, metaxylem area and phloem) was the highest in *C. brownii* at low elevation. Vascular tissues facilitate the water and solutes translocation from soil to the aerial parts of plants (Ali *et al*., 2009). Vascular bundle size is often linked to size of metaxylem vessels and phloem elements that are crucial for absorption and movement of water and photosynthates (Lucas *et al*., 2013). The proportion of leaf thickness was the minimum in *C. brunnea* from high elevation. Temperature is a major factor that causes changes in leaf structural traits at the elevation gradient. Leaf became thinner and fibrous, i.e., increased sclerification at high elevation, which protected plants from cold induce damages under unfavorable climate by folding, hence are less exposed to external environment (Ahmad *et al*., 2016). The survival of plant species colonizing high elevations primarily depends on the strength of sclerification. The proportion of dermal tissue was increased in *C. brownii* at highest elevation. Epidermal thickness acts as a barrier to external environment, which is essentially important for survival under adverse climatic conditions (Chourasia, 2017).

Principal component analysis: Plant height and leaf area of *C. brownii* was linked to high elevation, which was influenced by soil ECe, total soluble salts and inorganic ions like Ca^{2+}, Mg^{2+}, and K^{+}. In *C. brunnea* at high elevation, shoot length, inflorescence length and plant fresh weight were associated with environmental traits like maximum temperature, wind speed and relative humidity, and soil factors like ECe, total soluble salts, Cl^{-}, Ca^{2+}, Mg^{2+}, SO_4^{2-}. Nutrient availability along with suitable temperature and relative humidity is an ideal condition for growth and development of high mountainous species (Ahmad *et al*., 2016).

Stem radius and metaxylem area in *C. brownii* were co-occurred with soil ECe, total soluble salts and inorganic ions (K^{+}, Ca^{2+}, Mg^{2+} and SO_4^{2-}). Root traits in *C. brownii* and root and stem traits in *C. brunnea* showed no association with soil or environmental characteristics at high elevation. Increased stem area is related to better storage of solutes, while metaxylem with easier conduction of nutrients (Ali *et al*., 2009). Leaf traits like midrib thickness, bulliform thickness, abaxial epidermal thickness and abaxial stomatal density in *C. brownii* at high elevation were influenced by soil ECe, total soluble salts and inorganic ions like K^{+}, Ca^{2+}, Mg2+ and SO_4^{2-}. Thickened leaves and epidermis with stomata oriented on abaxial leaf surface is linked with water conservation, as reported by Fatima *et al*., (2022). Leaf attributes in *C. brunnea* did not show any association with soil parameters at high elevation.

Conclusion

It was concluded that adaptive components along elevation gradient varied greatly, which were specific to individual species. Modifications for cold tolerance in *C. brownii* at high elevation was linked to increase in shoot length, inflorescence length and leaf area. Root traits were increased epidermis and endodermis thicknesses and metaxylem area. In leaf internal structure, an increase was observed in midrib thickness, abaxial epidermal thickness, aerenchymatous area, bulliform thickness and adaxial stomatal density. *Carex brunnea* exhibited increased root length and leaf area at high elevation. Among stem traits, this species showed increased cortical region thickness. Leaf modifications at high elevation were adaxial and abaxial epidermal thickness, and adaxial epidermal cell area. Such modifications guaranteed *Carex* spp. for successful acclimation in extremely cold environments at high elevations.

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