Assessing the potential of anatomical characters for the circumscription of species of *Ephedra* in India

Prabha Sharma, Pradeep K. Bhandari, Anshul Dhyani and Prem L. Uniyal¹

Department of Botany, University of Delhi, Delhi 110007, India

¹Corresponding author, e-mail: uniyalpl@rediffmail.com

[Received 17.05.2020; Revised 25.06.2020; Accepted 27.06.2020; Published 30.06.2020]

Abstract

Various morphological details have been studied to configure the liable differences among the populations of *Ephedra* present in different altitudes of Lahaul and Spiti regions of India. Populations were distinguished on the basis of size of plants, colour of bark, branching pattern, number of scale-leaf and presence of papillation on the scale leaf, length of the internodes, and differences in the fruiting bodies. Epidermal features such as cell shapes, cuticular ornamentation and stomatal distribution, variations in the size of stomata, presence or absence of aborted stomata, stomatal index, shape and size of pore, size of lamellae, extent of papillation, presence or absence of pit have been observed in the cuticle. This study may help in circumscription of various species of *Ephedra* and distinguishing species on the basis of morphological variations found in the collected populations.

Keywords: *Ephedra*, Cuticle, Morphology, Stomatal frequency, Systematics

INTRODUCTION

Ephedra Tourn. ex L. (Ephedraceae) is widely distributed, equally in the temperate and subtropical regions of semiarid and desert conditions in high altitudes. Plants are dwarf, xerophytic, woody sub-shrubs, or herb, rarely exceeding 2 m in height. A long tap root occurs in xerophytic species. Stems are often profusely branched, having green branchlets arranged opposite or in whorls at nodes. Shoots are terete and articulate, while the internodes characteristically have numerous longitudinal striations. The epidermal cells are highly cuticularized except in the region of stomata which are located in the furrows in vertical rows. The elongated internodes are the result of intercalary meristems, which is present above each node. The branches arise from the axil of minute scale-leaves, which have parallel venation and borne in pair at each node. Scale leaves are opposite-decussate manner, remain united at their base to form a basal sheath and are deciduous. The young stems are green-phyllode and perform photosynthesis. Male strobilus is organised like an inflorescence, axillary, arising in whorls. The female strobilus arises in whorls of 2 – 4 and consists of a central axis with 2 – 4 or more paired opposite-decussate bracts.

Some species of *Ephedra*, such as *E. sinica* Stapf, *E. gerardiana* Wall. ex C.A. Mey., *E. major* Host (= *E. nebrodensis* Tineo), *E. intermedia* Schrenk & C.A. Mey. and others, have traditionally been used by indigenous people for a variety of medicinal purposes, including the treatment of asthma, hay fever, rashes of allergic origin and the common cold. Goats and yaks feed on its branches during winter (Porwal *et al.* 2003). Its dried twigs yield alkaloids (ephedrine and pseudoephedrine) which are used as drugs (Caveney *et al.* 2001; Chauhan 1999). These compounds are sympathomimetics with stimulant and decongestant qualities and are related chemically to the amphetamines.

Epidermal characters have received wide recognition in the classification of gymnosperms. Kerp (1990) studied fossil gymnosperms by taking in consideration of the

cuticular characters and stated that cuticles are the only source of information for the fossil plants. Cuticular analysis is a valuable method for the identification, classification and correlation of potential taxonomic studies. Florin (1931) and Harris (1932) initiated cuticular studies with special emphasis to the morphology of stomata. Salisbury (1927) defined the ecological significance of stomata and stomatal frequency which forms a salutation contribution to this deserted ground of ecological inquest. Stomatal characters such as stomatal frequency, stomatal density and stomatal index have also been used as an indicator of CO₂ concentration (Chen *et al.* 2001; Royer 2001; Kouwenberg *et al.* 2002). Thomas & Bancroft (1913), Florin (1931, 1936), Harris (1932, 1969) and Pant & Nautiyal (1963) emphasized the significance of the epidermal characters in demarcating different taxa of gymnosperms. The epidermal structure of *Ephedra major* subsp. *procera* (C.A.Mey.) Bornm. (*Ephedra nebrodensis* var. *procera*), *E. major* Host. (*E. villarsii* Gren. & Godr.); and *E. foliata* Boiss. ex C.A. Mey. had been described by Pant and Mehra (1964) and Pillai & Chacko (1979). Pant & Verma (1974) performed a detailed study on this aspect with living and herbarium specimens of *Ephedra*. Pant & Mehra (1964) examined the ontogeny of typical haplocheilic stomata in the leaves and stems of *Ephedra foliata*. Inamdar & Bhatt (1972) described the structure of epidermis and stomatal development in the stem leaf and cones of *Ephedra foliata* and *Gnetum ula*. Indeed there is a need to study cuticular structures of the populations at various habitats and altitudes.

A survey of stem anatomy of Gnetales at the species level has been undertaken in order to build up novel information of systematic and phylogenetic values. Bark and axis anatomy of *Ephedra*, *Gnetum* and *Welwitschia* were summarized by Pearson (1929) and Martens (1971) and elaborated the anatomical studies on *Ephedra* by Carlquist (1975, 1992, 2001). Tiagi (1966) investigated the vascular anatomy of *Ephedra foliata* and considered the primary vascular system as unit system being perforated, ectophloic dictyostele in contrast to the earlier observations of Marsden & Steeves (1955) who interpreted it as eustele where internodal bundles are incessant with leaf-traces. Cressen & Evert (1993) have given a very daunting account of vasculature in the stem of *E. viridis* Coville. Behnke & Paliwal (1973) reported only two cell types in the phloem of *E. campylopoda* C.A.Mey. *Ephedra gerardiana* Wall. ex Stapf. and *E. rupestris* Benth. are nearly devoid of vessel and grow in higher elevations than do other species of *Ephedra* (Hunziker 1949; Riedl 1963). Tracheids of *Ephedra* have pit membranes similar to conifer species (Bauch *et al.* 1972; Dute *et al.* 2014). *Ephedra sinica* growing in the Mongolian steppe was found to have a low content of photosynthetic tissue with high photosynthetic efficiency and low rate of transpiration and to be extremely proficient in water use (Ivanov *et al.* 2004). Shorter tracheary elements are more adaptive in higher altitudes as it may be probably an adaptation to extreme draughts when plants become prone to severe embolism. *Ephedra* bears three kinds of tracheary elements, i.e. vessel elements, tracheids and fibre-tracheids (Motomura *et al.* 2007).

Looking at the immense importance of *Ephedra*, economically as well as ecologically, systematic studies are made on the populations of *Ephedra* collected from Lahaul and Spiti district of Himachal Pradesh. Epidermal features are very important as they are frequently used in various fields of science including taxonomy, pharmacology, forensic studies, identification of food and feed and adulteration, etc. Present study deals with external morphology, epidermal features such as cuticle along with the stem anatomy with consideration for the systematic studies of *Ephedra*. Epidermal features such as cell shapes, cuticular ornamentation and stomatal distribution, variations in the size of stomata, presence or absence of aborted stomata, stomatal index, shape and size of pore, size of lamellae, extent of papillation, presence or absence of pit have been observed in the cuticle. In the stem anatomy variations in the extent of papillations, thickness of cuticle, variations in epidermal cell shape, extent of fibres in the cortical region are taken as the important criteria

for characterization of the populations present at different altitude. This study may help in circumscription of various species of *Ephedra* and distinguishing species on the basis of morphological variations found in the collected populations.

MATERIAL AND METHODS

Area of study:

Figure 1. Map showing Area of Study: Himachal Pradesh- Lahaul Spiti region (Chandrashekhar *et al.* 2003; www.indiamap.com)

Lahaul and Spiti comprise steppe-like vegetation, with *Caragana*, *Artemisia*, *Hippophae*, *Ephedra* and *Arenaria*. Fairly bigger patches of *Ephedra* scrub were delineated in these arid cold deserts, usually in xerophytic formations. The annual rainfall in the region is about 5 mm. The maximum and minimum temperature is around 30.5 and –19.5° C respectively (Porwal *et al.* 2003). Total 11 different regions of Lahaul and Spiti district were taken for the systematic study of *Ephedra*, which are indicated by green dots on the map:

Lahaul

1. 4 km behind Sissu
2. Helipad
3. Purat
4. Kardong
5. Dharcha
6. Patseo

Spiti

1. Khurik (blue)
2. Khurik (green)
3. Mohrang (dark orange scale leaf)
4. Mohrang (transparent scale leaf)
5. Sumling

Plant Collection

Healthy twigs along with male and female strobilus were collected with the help of Tree Pruner and tagged with field numbers and were kept in the polythene bags. The field numbers which were given in the field were assigned as DU-(01786-0321) to DU-(01786-0450). The field data such as locality, habitat, and brief descriptions were recorded in the *Field Note Book* corresponding to the field numbers. For updated nomenclature www.plantsoftheworldonline.org has been consulted.

Preservation of Plant Material

Voucher specimens were processed, including HgCl₂ poisoning, into mounted Herbarium-sheets following conventional methods (Jain & Rao 1977). Some specimens were also fixed in FAA and preserved in 90 % ethanol. The mounted, accessioned and labelled herbarium sheets were stored in the wooden cupboard in which small packets of naphthalene and p-dichloro-benzene mixture was kept in the ratio of 3:1 which acts as insect repellent. The specimens were deposited in the Herbarium of the Department of Botany, University of Delhi, Delhi (DU-01786-0321 to DU-01786-0450).

Slide preparation

Stem Cuticle: Cuticle was separated from the internodal regions following modified method of Ghouse & Yunus (1972). The material was macerated by in 60 % HNO₃ for 3 – 4 hours. The cuticle was then peeled off with the help of needle and cleared with the help of brush and permanent slide of cuticle was prepared using Saffranine stain in ethanol dehydration graded-series and mounted in DPX showing both surfaces of cuticles separately. Slides were then observed under microscope and different measurements were taken with the help of ocular micrometer. Different aspect recorded include stomata size, cell size, size of polar lamellae, lateral lamellae, papillations, number of columns etc., both of ridge and furrow regions and presented in tabular form.

Microtomy

For Microtomy small pieces of fixed inter-nodal region of the stem was taken in glass vial and passed through the tertiary butyl alcohol grades and finally embedded in wax. Blocks were prepared by pouring the material with pure wax in the circular glass plate on the hot

plate (applied glycerine before pouring the wax in the circular glass plates). Ribbons of 0.8 µm thickness were cut and mounted on slides thinly smeared with 1 % formalin and gum solution. The sections were stained with safranin using ethanol grades and finally mounted in DPX (O' Brien & McCully 1981)

Photography

Photographs in field and of mounted herbarium-specimens were taken by Sony Digital camera (DSC-W55). Morphological characters were studied under a Stereo-binocular microscope (Bausch and Lomb, model no. 6V15). And, micro-photographs were taken by Sony Digital camera model no. VX DSC- W7.

RESULTS AND DISCUSSION

The present work incorporates various populations of *Ephedra* spp. collected from the different localities belonging to the Lahaul and Spiti districts of Himachal Pradesh to find out the possible variations with the help of morphological and micro- morphological investigations. The range of Western Himalayas earlier thought to encompass the species *E. intermedia* in dominance. However, the current studies after a long gap of 70 years revealed the recognition of 4 new species [*Ephedra kardongensis* sp. nov.; *Ephedra khurikensis* sp. nov. (Blue); *Ephedra khurikensis* sp. nov. (Green); *Ephedra sumlingensis* sp. nov. and *Ephedra pangiensis* sp. nov.] and 7 populations with distinct morphological characters (Sharma & Uniyal 2008, Sharma *et al.* 2010, 2014, 2015; Sharma & Singh 2015). This was possible due to the availability of reproductive parts as the authenticity of identification of different species depends much on conservative and genetically controlled morphology of reproductive parts.

The populations of *Ephedra* observed in different habitats, 4 types of plant-habit was recognized, i.e. small erect shrub, medium erect shrub, bushy erect shrub and tufted erect shrub. Their color varied from dull green! light green! very light green! dark green! very dark green! very dull green! blue. Marked variation in inter-nodal length and diameter was also observed. Populations of Khurik (Blue) showed a very characteristic bluish ting which was not observed anywhere else. Similarly, populations of Mohrang (DOSL and TSL) showed distinctive dark orange scale leaves and transparent scale leaves which was again not observed in any other population. Reproductive structures were also analyzed and variations among the populations were studied and marked differences in shape and size of male strobilus, pairs of bract, size of bract and length of synangiophore with synangia were analyzed. Similarly, some extent of variations were also observed in shape and size of female strobilus, pairs of bracts, size of bract, nature and length of opening of micropyle (Table 1a, b).

Number of columns on ridge-region is found lesser than that of the furrow-region. Shape and extent of papillae varied drastically in various populations. Papillations present in the population of Spiti region was much more prominent than that of Lahaul population. Pits, stomata size, pore size, polar lamellae, contiguous stomata, aborted stomata and stomatal index of the populations present in Lahaul and Spiti showed marked difference (Table 2a, b).

The outline of T.S. of young internode was average to distinctly wavy due to the variation in the presence of ridges and furrows which runs longitudinally on stem surface . A distinct, heavily cutinised (ca 9.0 - 10.6 µm thick, (Figure 2A,B) epidermis in all the populations were recorded, however, amount of papillose projections varied. Epidermis consists of a single row of thin walled square - oval shaped cells in most of the populations. In the furrow region it is pierced by stomata, which are usually one-cell deep and have a

Table 1a: Over all mean values for various morphological attributes of *Ephedra* sampled from Lahaul

Locality	4 Km behind Sissu	Helipade	Purat	Kardong	Dharcha	Paseo
Character states						
Habit						
Habitat						
Stem						
A. Colour	Small, erect shrub (30cm)	Medium erect shrub (50- 60cm)	Small erect shrub (20cm)	Bushy erect shrub (1.0m)	Bushy erect shrub (70- 90cm)	Tufted erect shrub (10- 15cm)
B. Internodal length	Gentle rocks	Open ground	Entirely muddy hills	Gentle slopes	Steep slopes	Huge rocks
C. Diameter						
Leaf						
A. Shape	Dull green	Light green	Very light green	Dark green	Very dark green	Very dull green
B. Length	1.0- 2.0cm	2.5-3.0cm	0.5- 1.8cm	1.0- 2.0cm	2.0- 2.5cm	0.5- 1.5cm
C. Colour	0.5- 2.0mm	0.5- 2.5mm	0.5- 2.5mm	0.5- 2.0mm	0.5- 2.0mm	0.5- 2.0mm
Flower						
A. Shape	Triangular, acute tip	Triangular, acute tip	Triangular, acute – obtuse tip	Triangular, acute - obtuse tip	Triangular, obtuse tip	Triangular, obtuse tip
B. Length	1.5mm	2.0mm	1.5mm	1.5mm	2.5mm	1.0mm
C. Colour	Membranous, white	Transparent, brown base	Transparent, white base	Transparent, brown base	Transparent, brown base	Transparent , white base
D. Connation	1/2 connate	3/4 connate	1/2 connate	1/2 connate	1/2 connate	1/2- 3/4 connate
Male strobilus						
A. Shape and Size	ABSENT	ABSENT	ABSENT	Broadly ovoid, 3.0x 2.5mm	Broadly ovoid, 3x2 mm	Ovoid, 2.5x2.0mm
B. Pair of bracts	Round - ovoid, 3.0x 2.0mm			3	5	3
C. Size of bract	4			1.0- 2.5x1.0- 1.5mm	2.0- 2.5x1.5 -2.0mm	1.0- 2.0x1.5 -2.0mm
D. Length of synangiphore with synangia	1.0- 2.0x 1.0- 1.5mm			3.0mm	3.0mm	4.0mm
Female strobilus						
A. Shape and Size	ABSENT		ABSENT		ABSENT	ABSENT
B. Pair of bracts		Broadly elliptic, 6x5mm		Elliptic, 7x 5mm		
C. Size of bract		4		3		
D. Colour of seed		2.0- 5.0x 2.0-4.0mm		3.0- 6.0x 2.0- 4.0mm		
E. Size of seed		Brown		Brown to black		
F. Nature of micropyle		4.0x 2.0mm		3.0x 2.0mm		
G. Length and opening of micropyle		Highly coiled		Straight		
		2.5mm, oblique		2.0- 4.0mm, round		

Table 1b: Over all mean values for various morphological attributes of *Ephedra* sampled from Spiti

Locality → ↓Character states	Khurik (Blue)	Khurik (Green)	Mohrang (DOSL)	Mohrang (TSL)	Sumling
Habit	Medium erect shrub (40 – 50cm)	Medium erect shrub (40 – 50cm)	Medium erect shrub (30 – 40cm)	Medium erect shrub (30- 40cm)	Medium erect shrub (50 – 60cm)
Habitat	Open ground	Open ground	Open ground	Open ground	Open ground
Stem					
A. Colour	Blue	Green	Light green	Dark green	Very light green
B. Internodal length	3.0- 4.0cm	2.5- 3.0cm	3.0- 4.0cm	3.0- 3.5cm	2.0- 4.5cm
C. Diameter	0.5- 3.0mm	0.5- 3.0mm	0.5-2.5mm	0.5- 2.5mm	0.5- 2.0mm
Leaf					
A. Shape	Triangular, acuminate tip 2.0mm	Triangular, acuminate tip	Triangular, acute tip	Triangular, acute tip	Triangular, acuminate tip
B. Length	Transparent, brown base	3.5mm	5.0mm	3.0mm	4.5mm
C. Colour	½- ¾ connate	Transparent, chocolaty base ¾ connate	Dark orange ¾ connate	Transparent, slight orange base ¾ connate	Membranous, brown base ½- ¾ connate
D. Connation					
Male strobilus					
A. Shape and Size	Ovoid, 7.0x 3.5mm	Broadly ovoid, 3x 2.5mm			Elliptic, 8.0x 4.5mm
B. Pair of bracts	5	3			5
C. Size of bract	1.5 -2.5 x 1-2mm	1-2x1-1.5mm			1.0- 3.0x 1.0- 2.0 mm
D. Length of synangioophore with synangia	4.0mm	3.0mm			5.0 mm
Female strobilus					
A. Shape and Size	Elliptic, 7.0x 4.0mm	Elliptic, 9.0x 5.0mm	Elliptic, 8.0x 5.0mm	Elliptic, 6.0x 4.5mm	Elliptic, 6.0x 4.0mm
B. Pair of bracts	3	3	3	3	4
C. Size of bract	1.0- 5.0x 1.5- 4.5mm	1.0- 6.0x 3.0- 5.0mm	2.0- 6.0x 1.5- 5.0mm	1.5-5.0x 1.5- 3.0mm	3.0-6.0x 3.0- 4.5mm
D. Colour of seed	Black	Brown to black	Brown	Maroonish black	Maroonish brown
E. Size of seed	5.0x 2.0mm	4.0x 2.0mm	5.0x 2.0mm	4.0x 2.0mm	5.0x 2.5mm
F. Nature of micropyle	Coiled	Coiled	Coiled	Coiled	Coiled
G. Length and opening of micropyle	3.0mm, oblique	2.0mm, fringed and oblique	5.0mm, round to oblique	4.0mm, oblique	3.0mm, round

Table 2a. Micro-morphological details of Western Himalayan *Ephedra*: (Lahaul)

Regions → Characters ↓	4 km behind Sissu	Helipade	Purat	Kardong	Dharcha	Patseo
Epidermal cells (Shape)	Rectangular	Small, rectangular	Small, rectangular	Rectangular-square	Barrel shaped	Small, barrel shaped
No. of columns in ridge region	2 – 7	2-5	2- 4	2- 7	2- 9	2- 8
Papillation (Shape and extent)	Short local buldge; + + +	Circular buldge; + + +	Circular patch; +	Circular patch; + +	Small circular patch; + +	Buldge; + + + +
Pits (Extent)	+ + +	+ + +	+ + +	+ + + +	+ + + + +	+ + + + +
Stomata Size(LxB)	40.0× 15.0 µm	38.0× 18.0 µm	33.0× 15.0 µm	32.0× 16.0 µm	37.0× 19.0 µm	32.0× 16.0 µm
Pore Size(LxB)	9.0× 3.0 µm	11.0× 3.0 µm	10.0× 3.0 µm	9.0× 3.0 µm	9.0× 3.0 µm	7.0× 3.0 µm
Polar lamellae Size(LxB)	12.0× 3.0 µm	14.0× 3.0 µm	11.0× 3.0 µm	12.0× 3.0 µm	13.0× 3.0 µm	12.0× 4.0 µm
Extent of contiguous stomata	+ + +	+ + + +	+ + + +	+ + +	+ + + + +	+ +
Number of columns in furrow region	6- 12	4- 17	8- 16	5- 15	4- 14	3- 15
Aborted stomata (Extent)	+ + + + +	+	+ +	+ + +	+ + +	+ + + +
Stomatal Index	24	23	19	22	19	18

+ + + + + Excellent; + + + + Very good; + + + Good; + + Average; + Below average

prominent sub-stomatal cavity. Layers of cortical cells varied from 3 to 7 with thin walled chlorenchymatous cells differentiated into elongated palisade cells (2 layers) and oval- circular spongy cells (3 layers) (Figure 2A,B). Calcium oxalate crystals are present in very good amount in the cortical region in all the populations. Populations were also distinguished by the presence of fiber-bundles, which were located generally in four different regions and accordingly designated subepidermal (under the ridges), cortical, pericyclic and medullary fiber-bundles. Fiber cells are penta- to hexa-gonal, non-lignified but thick walled with obliterated lumen. Fiber-bundles are present in very good amount below the ridge region and present excellently all over the cortical region. Pericycle fibers occur as discontinuous bundle caps (Figure 2 E,F). Vascular bundles are conjoint, collateral and open; xylem is triangular and pointed towards the pith. The variation in the amount of Pith is also recorded, that occupies a large central part of the internode and is composed of polygonal and large rounded cells (Figure 2 G,H). The central cells are larger in diameter and filled up with very good amount of dark brown sticky resinous substance (Figure 2G, H).

The epidermal peel of internode shows discrete stomatiferous and non-stomatiferous bands in all the populations. The epidermal cells are rectangular arranged in longitudinal rows. Surface papillae and pits varied in populations which were found located on the cells of non-stomatiferous band in good amount. Papillae present in the non-stomatiferous regions of the cuticle in the form of short local buldge; in some cells papillae were not very prominent and are present in the form of circular patches (Figure 2 E,F). Number of columns in the ridge region varies from 2 – 7.

In the furrow region stomata are arranged in longitudinal rows and those of the adjacent rows are usually alternating, or sometimes contiguous (Figure 2 I,J). The stomatal apparatus is haplocheilic and monocyclic. Generally stomata are of tetracytic type being typically surrounded by a ring of four subsidiary cells (two polars and two laterals). The polar subsidiaries are typically elongated in the radial direction, while the laterals are extended tangentially and their

Table2b. Micro-morphological details of Western Himalayan *Ephedra*: (Spiti)

Regions Characters ↓	Khurik (blue)	Khurik (green)	Mohrang (DOSL)	Mohrang (TSL)	Sumling
Epidermal cells (Shape)	Small, rectangular	Rectangular	Long, rectangular	Rectangular	Rectangular-square
No. of columns in the ridge region	2- 4	2- 6	3- 6	3- 7	4- 7
Papillation (Shape and extent)	Buldge; + + + + +	Buldge; + + + + +	Buldge; + + + + +	Buldge; + + + + +	Buldge; + + +
Pits (Extent)	+ + + +	+ + +	+ + + + +	+ + + +	+ + + + +
Stomata Size(LxB)	33.0× 15.0 µm	36.0× 14.0 µm	39.0× 19.0 µm	38.0× 15.0 µm	41.0× 18.0 µm
Pore Size(LxB)	9.0× 3.0 µm	9.0× 3.0 µm	11.0× 3.0 µm	12.0× 3.0 µm	12.0× 3.0 µm
Polar lamellae Size(LxB)	13.0 × 3.0 µm	13.0× 3.0 µm	14.0× 3.0 µm	13.0× 3.0 µm	15.0× 5 µm
Extent of contiguous stomata (Extent)	+ + +	+ + + +	+ + +	+ +	+ + + +
Number of columns in the furrow region	3- 9	4- 13	5- 13	5- 18	5- 14
Aborted stomata (Extent)	+ + + +	+ +	+ +	+ +	+ + +
Stomatal Index	24	23	21	22	22

+ + + + + Excellent; + + + + Very good; + + + Good; + + Average; + Below average; DOSL- Dark orange scale leaf; TSL- Transparent scale leaf

side towards the stomatal pore is slightly thickened. Contiguous stomata are present in good and aborted stomata are present in excellent amount. Guard cells showing polar and lateral lignin lamellae. In most of the populations the stomata is elliptic, ca. $40.0 \times 15 \pm 0$ µm. The pore size of stomata is ca. 9.0×3.0 µm; and the polar lamella ca. $12 \pm 0 \times 3.0$ µm. Number of columns in the furrow region varies from 6 - 12. Stomatal index is 24.

Thickness of cuticle also showed marked variation with Sumling population showing maximum thickness of 14 ± 0 - 15 ± 5 µm and of Kardong showing minimum thickness of 9 ± 0 - 10 ± 5 µm. Helipad population showed excellent wavy margin amongst all. Populations of Dharcha and Patseo showed excellent quantity of ridge and furrow region and also the excellent amount of fibres were present below the furrow region of the same populations along with the Khurik (Blue) region (Figure 2A,B). Populations from 4 km behind Sissu, Purat and Sumling showed excellent amount of fibres present all over the cortex. Number of layers in palisade and spongy parenchyma of different populations also showed marked variations. Population of Kardong, Khurik (Blue) (Figure 2B) and Mohrang (DOSL-Figure- 2 F and TDL- Figure 2J)) showed high amount of calcium-oxalate crystals in their cortical regions (Figure 2 B,C). No fibre was observed in the pith region of Lahaul populations but they were present in the pith region of Spiti populations (Figure 2 K, L). Pith was more or less large of all the samples (Lahaul and Spiti) and Kardong, Patseo and Mohrang (TSL) (Figure 2K,L) showed excellent amount of resinous substances (Table 3a, b).

Significance of systematic studies lies not only in the fact that we can construe the flora of a particular region but at the same time it gives us an idea of its biodiversity. It gives us the

Figure 2. A to D. Khurik Blue population of *Ephedra*: **A.** Overview of T.S. of stem; **B.** T.S. stem showing the details of cuticular epidermis and fibre bundles in the cortex; **C.** well-developed pith with resinous substance in the cells; **D.** Papillate epidermis and fibre bundles. **E** to **H** Moharang 1 population of *Ephedra*: **E.** Overview of T.S. of stem; **F.** T.S. stem showing the details of thick cuticle, thick walled epidermal cells and fibre bundles in the cortex; **G.** well-developed pith cells with resinous substance; **H.** Distinct Papillate epidermis, large fibre bundles and palisade like cells. **I** to **L** Spiti population of *Ephedra*: **I.** Overview of T.S. of stem; **J.** T.S. stem showing the details of thick cuticle, thick walled epidermal cells and abundance of fibre bundles in the cortex; **K.** well-developed pith cells with resinous substance; **L.** Distinct Papillae and large fibre bundles are in high number.

knowledge and skills needed to discover and utilize new natural resources, as knowledge of the variety and interrelationships of organisms has important applications in medicine, agriculture and industry. This kind of study also helps in bio-prospecting, discovery of biological indicators, sustainable agriculture and sustainable utilization of biological resources.

All the above mentioned results depicts variations among the populations which may be due to the speckled habitat and climatic conditions, such as extreme cold and dry arid atmosphere,

altitude variance etc. The populations were collected from various altitudes of Lahaul and Spiti which may be probable grounds for morphological, micro- morphological and anatomical variations among them. Altitude and atmospheric pressure also plays an important role in deciding the length/ height of the plants. At higher altitude generally plants attain less height to adapt themselves in the extreme cold environment. Scale leaves are also a measure which helps them in retaining water. Motomura *et al.* (2007) have concluded from their anatomical studies in *E. pachyclada* of Mustang district, Western Nepal that it showed adaptations to extremely dry conditions in high mountainous region. Fluctuations in amount of vessel formations were observed markedly. Moreover, several individuals at higher elevations completely lacked vessels and it's fluctuations were also observed in the individuals from lower elevation. Noshiro & Suzuki (2001), Liu & Noshiro (2003), and Jiménez-Noriega *et al.* (2017) have also described this type of adaptive variations in wood structures in dicotyledons at genera and species level. In some plants, as in *Cornus*, wood anatomical features showed clear latitudinal trends at genus level but this was not observed at the species level (Noshiro & Bass 2000). Bordered pits may be present exclusively, or intermixed with simple pits, or simple pits may be present exclusively on ray cell walls (Carlquist 2007). Bordered pits represent a way of maximizing wall strength while providing a maximal conductive area between cells (Carlquist 2001). Pits were also observed in almost all the populations of Lahaul and Spiti which slightly varied in their extent. Bordered pits among living ray cells are clearly common in Gnetales and woody angiosperms.

CONCLUSION

The differences in the epidermal characters suggest that the populations with different types of cuticle and epidermis belong to distinct species or varieties. These epidermal characters are constant throughout the genus. A novel and recent discovery of simple pits in the ridges and furrow regions of the stem cuticle are reported for the first time. It is inferred the each population of *Ephedra* is well adapted to its habitats through the variability of various morphological expressions which led the populations to occupy ample ecological range. Present study emphasizes that morphological characters equally provide information on the diversity and population biology and natural resources.

Acknowledgements

Authors are grateful to the Indian Institute of Science, Bangalore, Department of Biotechnology, New Delhi and the University of Delhi for the financial support, which made this study possible.

LITERATURE CITED

- Bauch, J.; Liese, W. & Schultze, R. 1972. The morphological variability of the bordered pit membranes in gymnosperms. *Wood Sci. Technol.* 6: 165 – 184.
- Behnke, H.D. & Paliwal, G.S. 1973. Ultrastructure of phloem and its development in *Gnetum gnemon*, with some observations on *Ephedra campylopoda*. *Protoplasma* 78: 305 – 319.
- Caveney, S., Charlet, D. A., Freitag, H., Maier- Stolte, M., & Starratt, A.N. 2001. New observations on the secondary chemistry of the world *Ephedra* (Ephedraceae). *Am. J. Bot.* 88: 1199 – 1208.
- Carlquist, S. 1975. *Ecological strategies of xylem evolution*. University of California Press, Berkeley, California.
- Carlquist, S. 1992. Wood, bark and pith anatomy of Old World species of *Ephedra* and summary for the genus. *Aliso* 13: 255 – 296.

- Carlquist, S. 2001. *Comparative wood anatomy*, 2nd ed. Springer Verlag, Berlin.
- Carlquist S. 2007. Bordered pits in ray cells and axial parenchyma: the histology of conduction, storage, and strength in living wood cells. *Bot. J. Linn. Soc.* 153: 157 – 168.
- Chauhan, N.S. 1999. *Medicinal and Aromatic Plants of Himachal Pradesh*, Indus Publishing Company, New Delhi.
- Chen, L.Q.; Li, C.S.; Chaloner, W.G.; Beerling, D.J.; Sun, Q.G.; Collinson, M.E. & Mitchell, P.L. (2001). Assessing the potential for the stomatal characters of extant and fossil *Ginkgo* leaves to signal atmospheric CO₂ change. *Am. J. Bot.* 88 (7): 1309 – 1315.
- Cressen, R.A. & Evert, R.F. (1993). Structure of the primary shoot of *Ephedra viridis* Cov. *Intn. J. Plant Sci.* 154: 264 – 279.
- Dute, R.R.; Bowen, L.A.; Schier, S.; Vevon, A.G.; Best T.L.; Auad, M.; Elder T.; Bouche, P. & Jansen, S. 2014. Pit membranes of *Ephedra* resemble Gymnosperms more than angiosperms. *LAWA J.* 35 (3): 217 – 235.
- Florin, R. 1931. Untersuchungen zur Stammesgeschichte der Coniferales und Corditales. I. Morphologie und Epidermisstruktur der Assimilationsorgane bei den rezenten koniferen. *K. Svenska Vetensk.Akad. Handl. Sr.* 10: 1 – 588.
- Florin, R. 1936. Die fossilen Ginkgophyten von Franz-Joseph-Land nebst Erörterungen über vermeintliche Cordaitales mesozoischen Alters. II. *Palaeontogr Abt B Palaeophytol* 82: 1 – 72
- Ghouse, A.K.M. & Yunus, M. 1972. Preparation of epidermal peels from leaves of gymnosperms by treatments with 40% HNO₃. *Stain Technol.* 47: 322 – 324.
- Harris, T.M. 1932. The fossil flora of Scoresby Sound, East Greenland. 2 Description of seed plants *Incertae sedis*, together with a discussion of certain Cyadophyta cuticle *Medd. Gronland* 85: 1 – 112.
- Harris, T.M. 1969. *The Yorkshire Jurassic Flora-III. Bennettitales*. British Museum (Nat. Hist.), London.
- Hunziker, J.H. 1949. Sinopsis de las especies Argentinas del genero “*Ephedra*.” *Lilloa* 17: 147 – 174.
- Inamdar, J.A. & Bhatt, D.C. 1972., Epidermal Structure and Ontogeny of Stomata in Vegetative and Reproductive Organs of *Ephedra* and *Gnetum*. *Ann. Bot.* 36: 1041 – 1046.
- Ivanov , L.; Ivanova, L.A.; Ronzhina, D.A.; Chechulin, M.L.; Tserenkhand, G. & Gunin P.D. 2004. Structural and Functional Grounds for *Ephedra sinica* Expansion in Mongolian Steppe Ecosystems. *Russ. J. Plant Physiol.* 51(4): 469 – 475.
- Jain, S.K. & Rao, R.R. 1977. *A Hand Book of Field and Herbarium Technique*. Today & Tomorrow’s Publication, New Delhi.
- Jiménez-Noriega, P.M.S.; Terrazas, T.; López-Mata, L.; Sanchez-Gonzalez A. & Vibrans, H. 2017. Anatomical variation of five plant species along an elevation gradient in Mexico City basin within the Trans-Mexican Volcanic Belt, Mexico. *J. Mt. Sci.* 14: 2182 – 2199.
- Kerp, H. 1990. The Study of Fossil Gymnosperms by Means of Cuticular Analysis. *Palaios.* 5: 548 – 569.
- Kouwenberg, L.L.R.; McElwain, J.C.; Kürschner, W.M.; Wagner, F.; Beerling, D.J.; Mayle, F.E. & Visscher, H. 2002. Stomatal frequency adjustment of four conifer species to historical changes in atmospheric CO₂. *Am. J. Bot.* 90(4): 610 – 619.

- Liu, J. & Noshiro, S. 2003. Lack of latitudinal trends in wood anatomy of *Dodonaea viscosa* (Sapindaceae), a species with a worldwide distribution. *Am. J. Bot.* 90(4): 532 – 539.
- Marsden, M.R.F. & Steeves, T.A. 1955. On the primary vascular system and the nodal anatomy of *Ephedra*. *J. Arnold Arbor.* 36: 241 – 258.
- Martens, P., 1971. Les Gnetophytes. *Handbuch der Pflanzenanatomie* 12(2): 1 – 295.
- Motomura, H., Noshiro, S., & Mikage, M. 2007. Variable Wood Formation and Adaptation to the Alpine Environment of *Ephedra pachyclada* (Gnetales: Ephedraceae) in the Mustang District, Western Nepal. *Ann. Bot.* 100: 315 – 324.
- Noshiro, S. & Baas, P. 2000. Latitudinal trends in wood anatomy within species and genera: case study in *Cornus* L. (Cornaceae). *Am. J. Bot.* 87: 1495 – 1506.
- Noshiro, S. & Suzuki, M. 2001: Ontogenetic wood anatomy of tree and subtree species of Nepalese *Rhododendron* (Ericaceae) and characterization of subspecies. *Am. J. Bot.* 88: 560 – 569.
- O' Brien, T.P. & McCully, M.E. 1981. *The study of plant structure principles and selected methods*. Termarcaphi Pvt. Ltd. Melbourne.
- Pant, D.D. & Mehra, B. 1964. Epidermal Structure and Development of Stomata in *Ephedra foliata* Boiss. *New Phytol.* 63(1): 91 – 95.
- Pant, D.D. & Nautiyal, D.D. 1963. Cuticle and epidermis of recent Cycadales leaves, sporangia and seeds. *Senckenb. Biol.* 44: 257 – 347.
- Pant, D.D. & Verma, B.K. 1974. Taxonomy of the genus *Ephedra*. Significance of stem and leaf epidermis and cuticle. *Bot. J. Linn. Soc.* 69: 287 – 308.
- Pillai, S. K. & Chacko, B. 1979. Nodal Diaphragm in *Ephedra foliata*. *Phytomorphology* 29: 13 – 15.
- Pearson, H. H. W. 1929. *Gnetales*. Cambridge University Press, Cambridge.
- Porwal, M.C.; Sharma, L. & Roy, P.S. 2003. Stratification and mapping of *Ephedra gerardiana* Wall. in Poh (Lahaul and Spiti) using remote sensing and GIS. *Curr. Sci.* 84 (2): 208 – 212.
- Riedl, H. 1963. Ephedraceae. In: K.H. Rechinger (ed.). *Flora Iranica*. Akademische Druck- und Verlagsanstalt. Graz, Austria. Pp. 1 – 8.
- Royer, D.L. 2001. Stomatal density and stomatal index as indicators of paleoatmospheric CO₂ concentration. *Rev. Palaeobot. Palynol.* 114: 1 – 28.
- Salisbury, E.J. 1927. On the Causes and Ecological Significance of Stomatal Frequency, with Special Reference to the Woodland flora. *Philos. Trans. Royal Soc. London.* Series B. 216: 1 – 65.
- Sharma, P. & Singh, R. 2015. A new species of *Ephedra* (Ephedraceae, Ephedrales) from India. *Phytotaxa* 218: 189 – 192.
- Sharma, P. & Uniyal, P.L. 2008. *Ephedra sumlingensis* Sharma & Uniyal (Ephedraceae) - a new species from Himachal Pradesh. *India. Bull. Bot. Surv. India* 50 (1-4): 1 – 6.
- Sharma, P.; Uniyal, P.L. & Hammer, O. 2010. Two new species of *Ephedra* (Ephedraceae) from the Western Himalayan region. *Syst. Bot.* 35 (4): 730 – 735.
- Sharma, P.; Uniyal, P.L. & Hammer, O. 2014. Phenotypic plasticity leading to sympatric speciation in populations of *Ephedra* in Lahul and Spiti (India). In: Sundaresan, J.; Gupta, P.; Santosh, K.M. & Boojh, Ram (eds), *Climte Change and Himalaya, Natural Hazards and Mountain Resources*. Scientific Publishers (India). Jodhpur. Pp. 205 – 217.

Sharma, P.; Uniyal, P.L.; Tripathi, S.B.; Negi, M.S. & Schwegendiek, A. 2015. AFLP based analysis of genetic diversity within Section Pseudobaccatae and Alatae of the genus *Ephedra* in Western Himalaya. *J. Innov. Biol.* 2(4): 245 – 249.

Thomas, H. H. & Bancroft, N. 1913. On the cuticles of some recent and fossil Cycadean fronds. *Trans. Linn. Soc. London.* (Ser. 2, Bot.) 8(5): 155 - 204.

Tiagi, Y.D. 1966. A contribution to the morphology and vascular anatomy of *Ephedra foliata* Boiss. *Proc. nat. Acad. Sci. India* 36: 417 – 436.

www.indiamap.com

www.plantsoftheworldonline.org