

Morpho-anatomical Studies of *Murraya koenigii* (L.) Spreng from Himachal Pradesh North-Western Himalayas

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ABSTRACT: *Murraya koenigii* (L.) Spreng commonly known as 'Curry Patta' in India belongs to the family Rutaceae. It is an important medicinal plant, which is mainly used in Ayurvedic and Unani formulations for the treatment of various diseases. The present study deals with the morphometric characterization, anatomical studies, palynological records and cytological analysis first time from various altitudinal ranges (300-2500 masl) of mid-Himalayan zone of Himachal Pradesh North-Western Himalayas. The morphological studies showed the average identification features of different accessions of *M. koenigii* species from various regions. A new morphotype had been reported from Mandi (854 masl) region with distinct phenotypic characteristics. Combined anatomical studies of leaf, stem and root were also executed and various characters were recorded to know more about the internal cellular organization. The new records and observations could help the future researchers to compile monographs, morpho-anatomical studies, pollen morphological analysis and cytological characterization for the studied taxa world-wide.

Keywords: *Murraya koenigii*, Rutaceae, Morphology, Anatomy.

INTRODUCTION

In the traditional systems, aromatic plants have taken different positions in health, spiritual and socio-cultural area of tribal and rural India. There are numbers of aromatic plants utilized for making herbal medicines and for other household purposes (Guleria and Vasisth 2009). Medicinal plants are groups of plants which acquire some special properties and these properties qualify them for drug ingredient, for medicinal purposes and act as therapeutic agents (Sharma *et al.*, 2010). India is included in one of the mega-biodiversity countries of the world, having medicinal, nutritional, ritual values (Rani *et al.*, 2013). In Himachal Pradesh, from 3500 known plant species, 500 are reported for their medicinal purposes (Singh and Thakur 2014). Himachal Pradesh have a special place in Vedic treaties due to its rich repository medicinal wealth (Sharma *et al.*, 2003). The genus *Murraya* containing fourteen species on global level; out of which two are found in India i.e., *M. paniculata* (L.) Jack and *M. koenigii* (L.) Spreng (Khosa and Prasad 1972). In these two species, *M. koenigii* is more popular because of its various medicinal and aromatic properties. Its leaves are widely used as a flavoring agent in numbers of food items (Dineshkumar *et al.*, 2012; Chauhan *et al.*, 2017). *M. koenigii* is known as Meetha neem or Curry patta in local language particularly in Indian subcontinent (Yankuzo *et al.*, 2011). Curry leaf history was seen in 1st - 4th century AD. The species generally distributed from Eastern and Southern Asia to Australia

(Narasimha *et al.*, 1975). The plant can be found in the moist forest ranges a height from 500-1600 meters, sometimes up to 1500-1655 m (Khosa and Prasad 1974; Khosa *et al.*, 1970). These regions are Bhutan, Guangdong, Vietnam, Pakistan, Shainan, Sri-Lanka, Laos, Yunnan (Xishuangbanna), Nepal, and Thailand (Jain *et al.*, 2012). It is a native of South Asian countries like India and Sri-Lanka mainly (Prakash and Natrajan 1974). *M. koenigii* commonly distributed throughout India and in many areas of the country now species has also been cultivated. It is abundantly found in Bengal, Sikkim to Garhwal, Assam, Cochin, Kerala, Western-ghats and Himachal Pradesh (Jain *et al.*, 2012, Gahlawat *et al.*, 2014). The species is found in almost all the districts of Himachal Pradesh viz., Bilaspur, Hamirpur, Shimla, Mandi, Kangra, Sirmour, Chamba, Una, Kinnour, Kullu and Solan (Chowdhury *et al.*, 1984; Bhardwaj and Seth 2017; Kumar and Duggal 2019). Plant shows many variations with respect to morphologically, anatomically and palynologically, but has not been documented properly till date. The gametophytic chromosome number of *M. koenigii* is 9 i.e., n=9 and sporophytic chromosome number is 18 (2n=18) (Sandhu and Mann 1988; Guerra *et al.*, 2000). The species has already been characterized on the basis of morphological and anatomical parameters, however very few studies are found on anatomy of the species *M. koenigii* from India. The present comparative studies have been made first time on germplasm of *M. koenigii* collected from various altitudinal ranges (300-2500

masl) of mid-Himalayan zone of Himachal Pradesh North-Western Himalayas to find out new morphotypes within the species.

MATERIAL AND METHODS

A. Plant Materials

The accessions of *Murraya koenigii* (L.) Spreng were collected from various altitudinal ranges such as, Una (435 masl), Bilaspur (673 masl), Kangra (733 masl), Hamirpur (790 masl), Mandi (854 masl), Chamba (1006 masl), Sirmour (1027 masl), Kullu (1278 masl), Solan (1550 masl) and Shimla (2206 masl) districts of Himachal Pradesh to study the intra-specific morpho-anatomical variations linked with altitudinal variations (Fig. 1). The various sites were visited in different seasons from 2021-2023 for collection of plant materials such as leaf, stem, fruit parts for morpho-anatomical characterization. The accessions numbers were given to each collected plant accession and were preserved properly in herbarium sheets for further research (Table 1). The voucher specimens of *M. koenigii* (L.) Spreng (MKU-101, MKB-102, MKK-103, MKH-104, MKM-105, MKC-106, MKS-107, MKK-108, MKS-109, MKS-110) were deposited in the herbarium of Department of Botany, Eternal University, Baru Sahib, Sirmour, (H.P.) India.

B. Morphological Studies

The average morphometric characters such as plant height (m), leaf width (cm), leaf length (cm), petiole length (cm), number of flowers per cluster, flower diameter (cm), flower color, inflorescence, sepals (number & size mm), petals (number & size mm), fruit color, fruit weight (mg), fruit diameter (cm), stem size (cm), and roots size (cm) were measured on different accessions (three from each selected sites) for all the collected germplasm of *M. koenigii* from various altitudinal ranges of mid-Himalayan zone of Himachal Pradesh. Based on morphological characters the results were used to determine the new morphotypes in it.

C. Anatomical Analysis

Mature leaves and stem parts were collected and fixed in FAA (Formalin acetic acid alcohol), these samples were dehydrated in tertiary butyl alcohol series for further use. Series of very fine, thin, transverse sections with the help of sharp blade were made gently for stem and leaf (mature and vegetative) parts of *M. koenigii* accessions from various selected study regions. Sections were stained with different dyes such as Safranin and Fast green combinations (Johansen, 1940). Micro-photographs of various stained sections were taken with the help of Co-axial trinocular research microscope.

D. Statistical Analysis

The data for statistical analysis was collected from average of three different experiments in each case and their mean, SD values were recorded by Microsoft excel.

RESULTS AND DISCUSSION

In the present study, morphological, anatomical, palynological and cytological characterization of various accessions of *M. koenigii* were studied. The accessions of *M. koenigii* were collected from various altitudinal ranges (300-2500 masl) of mid-Himalayan zone of Himachal Pradesh, North-Western India.

A. Morphology

Selection of different morphometric characters were deeply analyzed to identify and distinguish the new morphotypes within *M. koenigii* accessions. In the present study, on the basis of average morphometric characters new morphotype was also recorded with unique features. In all collected plants, the accessions from Mandi-854 masl (MKM-105) reported with unique characteristics as compared to other plant accessions from different altitude ranges of Himachal Pradesh and hence considered to be a new morphotype for *M. koenigii* species first time from Himachal Pradesh.

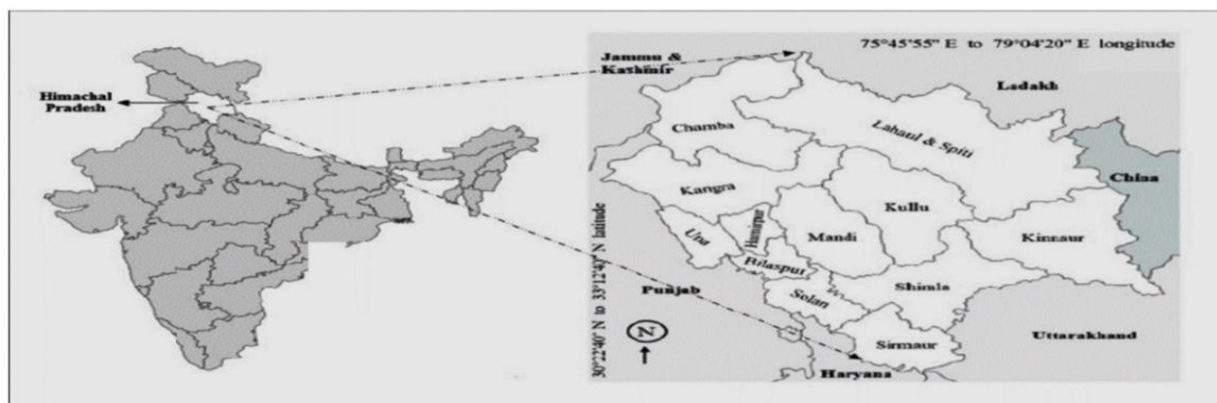


Fig. 1. Map of Himachal Pradesh showing various collection sites [Una (435 masl), Bilaspur (673 masl), Kangra (733 masl), Hamirpur (790 masl), Mandi (854 masl), Chamba (1006 masl), Sirmour (1027 masl), Kullu (1278 masl), Solan (1550 masl) and Shimla (2206 masl)] of *M. koenigii*. (Source: <https://mapchart.net>)

Table 1: Comparative average key morphometric characters across several accessions of the *M. koenigii* collected from various regions of Himachal Pradesh. (n=3).

Sr. No.	Morphometric Characters (<i>M. koenigii</i>)	Una (435 masl) (MKU-101)	Bilaspur (673 masl) (MKB-102)	Kangra (733 masl) (MKK-103)	Hamirpur (790 masl) (MKH-104)	Mandi (854 masl) (MKM-105)	Chamba (1006 masl) (MKC-106)	Sirmour (1027 masl) (MKS-107)	Kullu (1278 masl) (MKK-108)	Solan (1550 masl) (MKS-109)	Shimla (2206 masl) (MKS-110)
1.	Plant height (m)	3.07±0.04	3.96±0.57	2.67±0.04	3.71±0.19	4.94±0.06	3.48±0.30	2.95±0.06	2.24±0.08	3.38±0.16	3.07±0.13
2.	Leaf width (cm)	1.12±0.02	1.57±0.07	1.26±0.02	1.21±0.02	2.24±0.02	1.22±0.07	1.54±0.07	1.14±0.04	1.13±0.45	1.11±0.03
3.	Leaf length (cm)	2.48±0.03	2.96±0.05	2.53±0.20	2.40±0.08	4.46±0.15	2.29±0.07	3.15±0.28	2.14±0.04	2.26±0.10	2.31±0.03
4.	Petiole length (cm)	13.33±0.25	15.87±0.18	12.81±0.23	12.87±0.63	18.91±0.53	12.49±0.17	13.65±0.35	12.15±0.83	14.00±0.09	13.41±0.13
5.	Number of flowers per cluster	59.67±5.56	65.66±3.68	56.67±3.30	55.00±2.94	77.00±3.27	59.33±2.87	55.66±1.69	51.00±4.90	58.00±5.35	51.67±2.87
6.	Flower diameter (cm)	1.29±0.07	1.66±0.04	1.47±0.12	1.60±0.08	2.15±0.09	1.36±0.05	1.60±0.09	1.19±0.05	1.24±0.05	1.31±0.08
7.	Flower color	Whitish	Whitish	Whitish	Whitish	Whitish	Whitish	Whitish	Whitish	Whitish	Whitish
8.	Flower inflorescence	Terminal cyme	Terminal cyme	Terminal cyme	Terminal cyme	Terminal cyme	Terminal cyme	Terminal cyme	Terminal cyme	Terminal cyme	Terminal cyme
9.	Sepals (Number & Size mm)	Five- 5.51±0.03	Five- 6.00±0.01	Five- 6.00±0.03	Five- 5.77±0.26	Five- 8.63±0.20	Five- 5.54±0.28	Five- 6.0±0.12	Five- 5.49±0.04	Five- 5.60±0.03	Five- 4.91±0.95
10.	Petals (Number & Size mm)	Five- 7.45±0.06	Five- 6.87±0.08	Five- 7.67±0.18	Five- 7.76±0.17	Five- 9.70±0.18	Five- 7.54±0.24	Five- 6.0±0.16	Five- 7.40±0.07	Five- 7.63±0.33	Five- 9.0±0.16
11.	Fruit color	Green to purple	Green to purple	Green to purple	Green to purple	Green to purple	Green to purple	Green to purple	Green to purple	Green to purple	Green to purple
12.	Fruit weight (mg)	356.77±5.12	377.58±7.38	363.66±7.24	347.66±12.66	493.44±13.43	332.89±6.15	394.64±3.84	352.35±17.62	395.96±4.65	381.27±10.21
13.	Fruit diameter (cm)	1.15±0.05	1.50±0.02	1.31±0.06	1.47±0.21	2.45±0.10	1.14±0.04	1.88±0.02	1.13±0.05	1.15±0.05	1.16±0.05
14.	Stem diameter (cm)	4.12±0.04	5.31±0.23	4.86±0.10	3.94±0.07	8.22±0.28	4.08±0.03	4.05±0.03	3.87±0.15	4.03±0.13	3.79±0.23
15.	Root diameter (cm)	3.65±0.11	4.20±0.02	4.02±0.24	4.02±0.24	7.54±0.30	3.88±0.07	3.82±0.04	3.25±0.11	3.69±0.20	3.45±0.09

The major characters such as, plant height (4.94±0.06 m), leaf width (2.24±0.02 cm), leaf length (4.46±0.15 cm), petiole length (18.91±0.53 cm), number of flowers per cluster (77.00±3.27), flower diameter (2.15±0.09 cm), flower color (whitish-yellow), flower inflorescence (terminal cyme), sepals (number & size) (five, 8.63±0.20 mm), petals (number & size) (five, 9.70±0.18 mm), fruit color (green to purple), fruit weight (493.44±13.43 mg), fruit diameter (2.45±0.10 cm), stem size (8.22±0.28 cm), and roots size (7.54±0.30 cm) were recorded and found lots of variations as compared to other accessions (Table 1 and Fig. 2. A-I).

The leaves of the plant were green, reticulate and pinnate. Leaflets were presented with bearing 9-15

leaves on it. In *M. koenigii* leaves, a special type of aroma was there which considered it as an important aromatic plant. Young stems were light green in color which turns to dark green and then brownish when matured bearing several dots. Bark of the plant can be removed longitudinally, having white wood underneath. Flowering start from the middle of April and ends in the middle of May. The fruiting season was observed to continue from the middle of July to the end of August. Reticulate venation was presented; 9-15 on a leaflet. Green in color; aromatic, shiny, pinnate; having midrib with small hairs. These were bipinnately compound and exstipulate. Flowering season occurred in the months of March and April.

Flowers were of funnel shaped, white in color, small in size and had a characteristic fragrance which attract the pollinators; inflorescence was terminal cyme, which bears 30-90 flowers; actinomorphic, complete, stalked, ebracteate, bisexual, pentamerous, five lobed, calyx (5), stamens (10), petals (5), short style, inferior corolla, stigma bright and sticky, polyandrous androecium, long gynoecium, superior ovary. Fruits were shiny and

pulpy. Green in color when young then reddish and finally turns into black when mature.

These were round as well as oval in shape. Dicotyledonous seeds; one in each fruit or sometime two (02); rough surface, green in color when young then turned into cream color when mature. The roots of *M. koenigii* were dark brown to creamy white in color (Fig. 3. A.-L.).



Fig. 2. *M. koenigii* (L.) Spreng (A.) Young leaves (B.) Mature leaves (C.) Top view of leaves arrangement (D.) Mature stem (E.) Young floral buds (F.) Mature flowers (G.) Immature fruits (H.) Mature fruits (I.) Life cycle of flowers of *M. koenigii*.

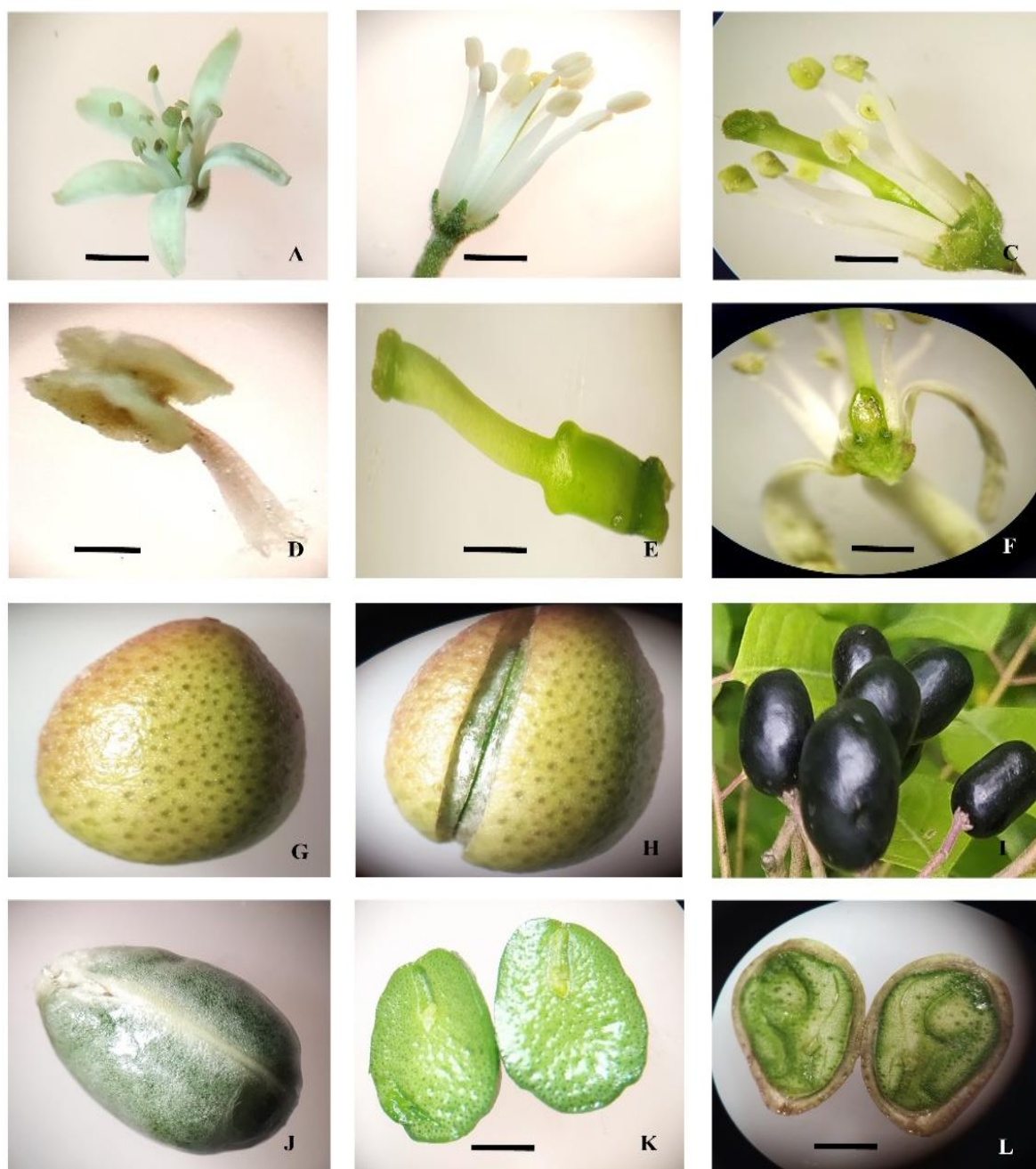


Fig. 3. *M. koenigii* (L.) Spreng (A.) Floral part (B.) Whorls of flower (C.) Androecium and gynoecium (D.) Anther and filament (E.) Stigma, style and ovary (F.) L.S. of gynoecium (G.) Young fruit showing small dots on outer surface (peel) (H.) Ripened fruit (I.) Removing of peel (J.) Thin covering on the inner surface of fruit (K.) Image showing dicotyledonous seed (L.) L.S. of fruit. (Scale bar = 10 µm).

One of the previous reports also indicating the similar characteristics such as, aromatic shrub or small tree, semi-deciduous, woody and slender stem (i.e., dark green to brownish in color), tree ranges a height up to 4.0-8.7 m (13-31ft.), reported brown to dark green color stem, small nodes like dots were presented, white color wood after peeling the bark (Sastri, 1949; Handral *et al.*, 2010). Leaves were having a special type of aroma which mentioned the presence of plant. Curry leaves were smooth and shiny, undersides were paler; pinnate,

reticulate venation, ovate, lanceolate, exstipulate (Gahlawat *et al.*, 2014; Gupta and Prakash 2009).

As per records, each leaf was containing 11-21 leaflets. Leaflets were containing short stalk, dotted glands, alternate and long petiole. Irregularly serrate leaf margins. Flowers were white in color, fragrant, small, funnel-shaped, stalked, ebracteate, regular, inferior, polypetalous, green corolla, pentamerous, polyandrous, stigma-bright and sticky; short style, complete, a terminal cyme, fully opened, presented in cluster, each

cluster having 60-90 flowers, calyx 5-lobed, petals-5 with length of 5.0 mm, stamen 10 in number, arranged in circles, gynoecium superior and long (size approximately-5.0-6.0 mm) (Prakash and Natarajan 1974). Fragrance of the flowers was sweet, self-pollinated, produces small size black berries. Appearance of seeds was shiny which were containing visible seeds (Narasimha *et al.*, 1975). Fruits were found in cluster, varies in number. These were ovoid and small sized, enclosed with the thin pericarp

(Sathaye *et al.*, 2012). Length of fruit was 1.4-1.6 cm and diameter was 1-1.2 cm; green in color (when young), turns purple black after the ripening; edible; containing a yellow volatile oil (Taha and Noorma 2008). As per the present study, a unique morphotype was reported within the accessions of *M. koenigii* species, and hence proved to be a new morphotype from mid-Himalayan zone of Himachal Pradesh.

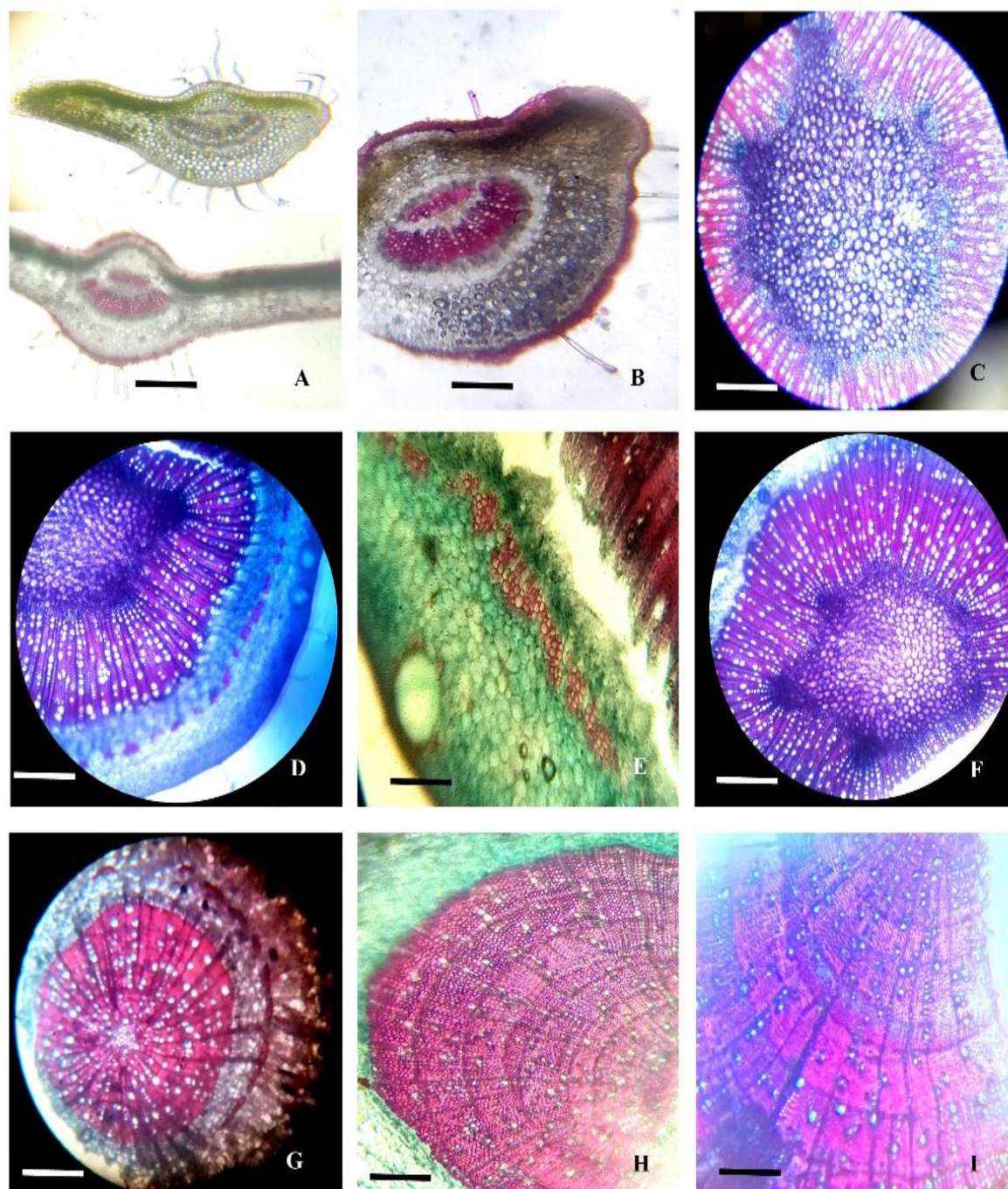


Fig. 4. *M. koenigii* (L.) Spreng (A.) T.S. of leaf (B.) T.S. of leaf showing vascular bundles (C.) T.S. of stem (D.) Cortex region of stem (E.) Enlarged part of cortex region (F.) T. S. stem showing pith and vascular bundles (G.) T.S. of root (H. & I.) Enlarged part of T.S. of root. (Scale bar — 10 μm).

B. Anatomy

Leaf: As per the anatomical studies, it was found that the uppermost layer was epidermis which was covered by cuticle from surroundings. Trichomes were present in the outer side mainly in the midrib region. Beneath this, collenchymatous cells with 1-4 layers were presented called as hypodermis. It was followed by polygonal and parenchymatous cells. Xylem and phloem were also shown in the leaf transverse section (Fig. 4. A.-B.). According to the literature, trichomes were found mostly in the midrib portion of upper surface than that of the lower surface, which corroborated with our own study. Epidermal region with rectangular cells, hypodermis and chlorenchymatous cells with several layers were also found in present study and similar results were also reported by Handral *et al.*, (2010) (Gupta and Prakash 2009). Studies revealed that the stomata were only found on the abaxial surface of the leaf and absent on the adaxial surface. Anomocytic type of stomata were found in leaf. Trichomes were more prominent in the midrib portion of upper surface than that of the lower surface (Soundappan *et al.*, 2018; Jarald *et al.*, 2008). Leaf transverse section reveals that the epidermal region was made up of rectangular cells and was followed by one to four (1-4) layers of hypodermis (collenchymatous) in carrying with two to five (2-5) layers of cells of chlorenchyma which were filled with the chlorophyll contents. Followed by ground tissues, which were made up of parenchymatous and polygonal cells crossover with vascular bundles. In that area, calcium oxalate (prismatic and sandy crystals) were also found. Phloem and xylem were made up of their own basic elements (Gupta and Prakash 2009; Jarald *et al.*, 2008; Yi and Wetzstein 2010).

Stem: In the T.S. of stem, thick cuticle covered the epidermis. Epidermis was single-layered, uniseptate and parenchymatous. Phloem cells and xylem was also seen in the section. Stem section cutting found that under the epidermal region oil glands were present and several trichomes were found on the outer side of epidermis which were unicellular and uniciliate. Epidermis was also encircled by the cuticle from surroundings. Compactly arranged 4-6 layers of polygonal and parenchymatous cells which formed the cortex. Xylem and phloem cells were also present, which was also found the studies of Jarald *et al.* (2008) (Yi and Wetzstein 2010). In the middle portion stem of *M. koenigii* formed protostele. Open, collateral and conjoint vascular bundles were presented. Protostele was recorded in the anatomical analysis of the stem of the plant, in which epidermis, vascular bundles and oil glands were also recorded (Fig. 4. C.-F.). Dotted oil glands, small hairs on outer surface; vascular bundles were present in vertical section in the stem of the plant. Epidermis was parenchymatous, monolayered, unicellular, uniseptate which in encircling by thick cuticle (Soundappan 2018; Jarald *et al.*, 2008). The diameter of epidermal cells was 7-8 to 15 μm . Epidermis also demonstrated the trichomes which were uniseriate and unicellular. There were oil glands (schizolysigenous) beneath the epidermal region.

Cortex region was constituted by parenchymatous and polygonal cells which were compactly arranged with 4-6 layers. Presence of sclerenchymatous cells (lignified) also showed in cortex region which was 30 to 50 μm . Xylem and phloem cylinders with medullary rays (bi or triseriate) consisted with the vascular system. These were open, conjoint and collateral. Total area of phloem and xylem was 38-95 and 73-119 μm , respectively. Thin wall of parenchymatous and polygonal cells constructed pith which contain starch grains (Yi and Wetzstein 2010; Pagariya, 2019).

Root: The cross-sectional contour of a thin lateral root was irregular. On the surface of the root was a black, thick, crushed epidermal layer. The five lobed xylem and thin phloem sheath of the large, vascular cylinder that made up the cortex. The round tap root was thin. Parenchyma cells in the inner cortex were compact, tiny, and polygonal. The thick-walled xylem fibres were arranged in radial lines. The cortex was made up of four to five layers, with the layer of phellogen between the outer and inner cortex being tangentially elongated and thin-walled. Long, narrow cylindrical cells called vessel elements had perforations on both ends. According to the studies there were very few reports regarding anatomy of *M. koenigii* (Gupta and Prakash 2009; Yi and Wetzstein 2010; Fahn, 2000). The present study gave the root, stem and leaf anatomical features in a combined form. Uneven cross-sectional outlines could be seen in thin lateral roots. The surface of the root bears a dark, thick covering of crushed epidermis. The vascular cylinder of the broad cortex had five lobes of xylem and a thin sheath of phloem. Circular tap roots were thin. Small, crowded, polygonal parenchyma cells made up the inner cortex. Secondary xylem cylinders had sclerotic pith and five or six primary xylem strands. The thick-walled, radial-lined xylem fibers of plants. In between the outer and inner cortex, there were four or five radially elongated, thin-walled layers of phellogen (Fig. 4. G.-I.). In previous study of powder microscopy, vessel components and parenchyma cells were clearly visible. The cells which made vessels were long, narrow, cylindrical, and perforated on both ends. The vessel components had a length of 180 to 280 μm (Fahn, 2000; Begum and Nath 2019).

CONCLUSIONS

Detailed comparative analysis were made first time on germplasm of *M. koenigii* collected from various altitudinal ranges of Himachal Pradesh Himalayas. Present study reveals that the *M. koenigii* is very common and frequently available in Himachal Pradesh. Its various other morphotypes with maximum biomass could also be reported from other locations. There are huge possibilities about availability of the new chemotypes and ecotypes as well. Therefore, for further future investigations the comparative studies with respect to phytochemistry, ecological variations and bioactivities must be linked morphotypes. So that, new morphotype or ecotype could be used properly in various industrial purposes to enhance the livelihood of local people of the Himalayan state.

FUTURE SCOPE

Future studies should focus on exploring the phytochemical diversity, ecological adaptability, and bioactivities of different *M. koenigii* morphotypes from varying altitudes. Such investigations may lead to the identification of novel chemotypes with potential industrial and pharmacological applications, contributing to sustainable utilization and livelihood enhancement in the Himalayan region.

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