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# Morpho-anatomical investigations of *murraya paniculata* (I.) Jack from the northhimalayan zones of Himachal Pradesh

# ANJALI THAKUR<sup>1\*</sup> AND VIVEK SHARMA<sup>2</sup>

<sup>1</sup>Department of Botany, Eternal University, Baru Sahib, Himachal Pradesh, India

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### **ABSTRACT**

In the current investigation, morphological and anatomical characteristics of many accessions of Murraya paniculata (L.) Jack from distinct Himachal Pradesh altitudinal ranges were examined. To verify the morphological changes, morphometric examinations of the plant sections, including the stem, leaves, flowers, and fruits, were conducted. Studying the blooms was also done with a zoom stereo microscope. Morphologically, the accessions varied greatly in terms of fruit shape and size, flower size, leaf color, shape and size and quantity. From the Sirmour region, a new morphotype with unique phenotypic traits has been described. Numerous interior cellular structures, including the number of layers of palisade cells and mesophyll and vascular bundles, were also discovered based on anatomical examinations. Future researchers may find it easier to create monographs, morpho-anatomical investigations for the taxa under study worldwide with the aid of the new data and observations.

**Keywords:** *Murraya paniculata*, morphology, anatomy

### INTRODUCTION

A class of plants known as medicinal plants has some unique characteristics that make them suitable as therapeutic agents, medication ingredients, and sources of medicine (Sharma et al. 2010). India has medicinal. nutritional, and ritual values, making it one of the world's mega-biodiversity countries and plant density is higher than ideal, competition for nutrients, light, and space may result in subpar plant growth (Rani et al. 2013, Thakur et al. 2015). Out of the 3500 recognized plant species in Himachal Pradesh, 500 are said to have therapeutic uses (Singh and Thakur 2014). Because of its extensive medical treasure reservoir, Himachal Pradesh holds a particular place in Vedic treaties (Sharma et al. 2003, Byomkesh et al. 2015). We have chosen Murraya paniculata (L.) for our study because it is one of the most significant plants in the field of medicinal herbs. This plant is a member of the Rutaceae (Citrus family), Genus Murraya (L.), which has about 150 genera and 1500 species (Yankuzo et al. 2011). In India, Murraya paniculata (L.) Jack is referred to as "Kamini" and orange jasmine. It is an evergreen shrub endemic to tropical and subtropical regions (Dosoky et al. 2016). It contains heartwood, which has a pale-yellow hue (Little and Woodbury 1974). Because orange iasmine is a hardy plant that can withstand a broad variety of soil conditions, it is often utilized as a hedge. Its leaves are also employed as a flavoring element in many Malay and Indian recipes because of its potent aroma (Ng et al. 2012). Chalcas paniculata L. and Camunium exoticum are synonyms for Murraya paniculata (Seidemann 2005). Its vernacular names are jasmine, satin-wood, orange jasmine, bun, mock orange, Naranjo, café de la India, honey bush, limoneria (Casado 2011). Common name in H.P. is Gandhela, curry patta (Kumar and Duggal 2019).

The cosmetic bark tree is another name for M. Paniculata (L.). Geographically, genus *Murraya* (L.) is most widely distributed throughout Asia and Oceania. This plant has significant therapeutic properties over its broad distribution region. It can be found in tropical regions with elevations between 1500 and 1655 metres above sea level (Gupta and Prakash 2009). It can be found in tropical and subtropical regions in Asia and Africa. It is widespread throughout India (Podder et al. 2011). According to Dosoky et al. (2016), M. paniculata (L.) Jack is indigenous to tropical and sub-tropical parts of the world, which include Taiwan, China, Nepal, Sri Lanka, India's northeastern states, and China. These days, M. paniculata is cultivated to

<sup>2</sup>Department of Phytomedicine, Baba Farid University of Health Sciences, Punjab, India; vivek03sharma@rediffmailcom, Corresponding Author: Anjali Thakur, Research Scholar, Department of Botany, Eternal University, Distt. Sirmour via Rajgarh, Himachal Pradesh, India, Pincode- 173101; anjalithakur.bot@gmail.com Mob: +91-98054-95104

flavor a variety of foods in a number of countries, mostly in Southeast Asia (India and Sri Lanka), the Pacific Islands, Australia, and Africa (Bonde *et al.* 2007). It is found throughout the Indian states of Assam, Himachal Pradesh, Bengal, and Kerala (Jain et al, 2012). In the Himachal Pradesh districts of Bilaspur (Barmana) (Sharma *et al.* 2010) and Hamirpur (Nadaun) (Jyoti *et al.* 2014), reports of *M. paniculata* have been made.

### **MATERIALS AND METHODS**

### **Plant Materials**

In order to investigate the intra-specific morpho-anatomical and cytological variations associated with altitudinal variations, accessions of Murraya paniculata (L.) Spreng were gathered from a variety of altitudinal ranges, including the districts of Sirmour (1027 masl), Hamirpur (790 masl), Mandi (854 masl), and Bilaspur (673 masl) in Himachal Pradesh (Figure 1: Table 1). From January to November 2021-2023, the different locations were visited during varying seasons in order to gather plant samples, such as immature buds for cytological investigations, leaves, stems, and fruit parts for morphoanatomical characterization, and flowers for palynological study. Each collected plant accession was assigned an accession number, which was then appropriately stored in herbarium sheets for future study (Table 1). M. paniculata (L.) Spreng voucher specimens (MPM-101, MPB-102, MPS-103, and MPH-104) were placed in the Department of Botany's herbarium at Eternal University, Baru Sahib, Sirmour, (H.P.) India.

## **Morphological Studies**

Morphological parameters were observed in the study locations to ascertain the extent of morphological heterogeneity in the collected accessions. The aim of this study was to identify new morphotypes in Himachal Pradesh by analyzing various morphometric characters, such as plant height (m), leaf shape-size (cm) and color; flower color, inflorescence, flower diameter (cm), sepals (number & size mm), petals (number & size cm), fruit color, fruit weight (mg), fruit diameter (cm), stem size (cm), and roots size (cm), based on these morphometric characteristics.

#### **Anatomical Studies**

For subsequent use, mature leaves and stem sections were collected and preserved in Formalin Acetic Acid Alcohol (FAA); the samples were then dehydrated in a tertiary butyl alcohol series. Using a sharp blade, a series of extremely thin and fine transverse sections were carefully cut for the mature and vegetative parts of the stem and leaves of *M. paniculata* accessions from different research regions. Different dyes, including Safranin and Fast green combinations, were used to stain sections (Johansen 1940). Using a co-axial trinocular research microscope, microphotographs of different stained slices were taken.

# **Statistical Analysis**

The average of three separate experiments per instance provided the data for statistical analysis, and Microsoft Excel was used to record the mean and standard deviation of each experiment.

# **RESULTS AND DISCUSSION**

In order to investigate the intra-specific morpho-anatomical and cytological differences associated with altitudinal variations, the present study examines the morphological, anatomical, and cytological characteristics of *M. paniculata* using accessions obtained from the several districts of Himachal Pradesh. These are Sirmour (Lana Machher, Baru Sahib); Hamirpur (Nadaun); Mandi (Ghagus); and Bilaspur (Chokhna, Ghumarwin, Jhandutta).



According to the literature, there aren't many publications on the morphology and anatomy of M. paniculata, particularly in the northern parts of India. This indicates that further



Figure 2. Murraya paniculata (L.) Jack showing huge morphological variations among (A-D) Mature Stem; (E-H) Leaflets; (I-L) Petals of flowers

research into it is necessary. Thus, by methodical research, the current study aimed to close these gaps. The *M. paniculata* accessions were gathered from across the mid-Himalayan zone of Himachal Pradesh, in different altitudinal ranges (673-1027 masl) (Fig. 1).

## **Morphological Studies**

In the current investigation, a new morphotype with distinctive traits was also recorded based on average morphometric characters. The accessions from Sirmour (MPS-1027masl) were found to have distinct features in comparison to other plant accessions from varying altitude ranges in Himachal Pradesh. As a result, they are thought to represent a new morphotype for the M. paniculata species, which has never been found in Himachal Pradesh before. Plant height (m), leaf length (cm), color, flower color and inflorescence, flower diameter (cm), sepals (number & size mm), petals (number & size cm), fruit color, fruit weight (mg), fruit diameter (cm), stem size (cm), and roots size (cm) are some of the major characteristics. When compared to other accessions, there were many differences in the height of plant (3.10±0.07m), breadth and length of leaves (2.17±0.03cm and 2.73±0.21 cm), and number of flowers per cluster (4.33±1.15), flower color

flower inflorescence (racemose), (whitish), number and size of sepals (five, 4.70±0.10 mm). number and size of petals (five, 1.87±0.15 mm), fruit color (dark green to orange), fruit weight (355.59±6.56 mg), fruit diameter (0.97±0.19 cm), stem diameter (3.96±0.04 cm), and roots size (2.87±0.14 cm) (Table 1). When young stems were light green in color, they eventually grew to dark green, then yellow, and finally light brown. It contained wood that was white and yellowish (Figure 2). The plant had pinnate, green, reticulate leaves. There were leaflets with nine to fifteen leaves on them. M. paniculata is regarded as an essential fragrant plant because of the unique type of aroma found in its leaves (Figure 2). The middle of March is when most flowers bloom, and fruiting season was seen to have begun in the middle of July and to continue until January to March. Reticulate venation was displayed on a pamphlet, numbers 3-9. Green in hue, fragrant, glossy, silky, and pinnate, with tiny hairs growing in the middle. These were exstipulate and bipinnately compound. The inflorescence was racemose, bearing four to seven flowers; the flowers were actinomorphic, complete, stalked, ebracteate, bisexual, pentamerous, five lobed, with a funnel-shaped, white or creamy white color, average size, and a distinct fragrance that attracted pollinators. The stigma was bright and sticky, and the flowers

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

Morphometric Characters ( <i>M.</i> paniculata)	Mandi (854 masl) (MPM-101)	Bilaspur (673 masl) (MPB-102)	Sirmour (1027 masl) (MPS-103)	Hamirpur (790 masl) (MPH-104)
Plant height (m)	2.77±0.15	3.07±0.08	3.10±0.07	3.04±0.03
Stem	Multi-stemmed	Multi-stemmed	2-3 Stemmed	Multi-stemmed
Leaf length (cm)	2.44±0.05	2.83±0.15	2.73±0.21	3.01±0.03
Leaf width (cm)	1.90±0.10	2.13±0.21	2.17±0.03	2.17±0.12
Number of flowers per cluster	3.33±0.58	4.33±1.53	4.33±1.15	4.67±1.15
Flower diameter (cm)	2.96±0.18	3.40±0.10	3.63±0.06	3.06±0.12
Flower color	Whitish	Whitish	Whitish	Whitish
Flower inflorescence	Racemose	Racemose	Racemose	Racemose
Sepals (No. & Size mm)	Five-	Five-	Five-	Five-
	4.23±0.12	4.57±0.15	4.70±0.10	3.77±0.15
Petals (No. & Size cm)	Five- 1.34±0.15	Five- 1.63±0.15	Five- 1.87±0.15	Five- 1.37±0.06
Fruit color	Dark green to orange	Dark green to red	Dark green to orange	Dark green to red
Fruit weight (mg)	341.15±2.91	377.53±1.84	355.59±6.56	348.01±7.53
Fruit diameter (cm)	0.81±0.05	1.13±0.21	0.97±0.19	$0.89 \pm 0.09$
Stem diameter (cm)	4.07±0.03	4.10±0.18	3.96±0.04	3.77±0.70
Root diameter (cm)	3.17±0.05	3.01±0.03	2.87±0.14	2.73±0.06

were polyandrous androecium, long gynoecium, and superior ovary. Fruits were dotted and glossy. When young, the colour is dark green; as it ages, it eventually turns orange or red. Most of these had a teardrop shape. Dicotyledonous seeds, cream-colored with a rough surface, one in every fruit (Figure 2, 3). The colour of M. paniculata roots ranged from light brown to creamy white. According to the previous studies, Kamat et al. (2015), the plant is aromatic, deciduous, evergreen, shrubby, or small tree that can reach a height of 6 to 9 metres and a range of 1500 metres. Its stem diameter is 13 centimetres. The number of leaves on a stalk alternates. glossy pamphlets; three to nine available. whole margins featuring cuneate apices and acuminate (pointed) tips. It has several stems that rise from the earth and are supported by lateral, tap, clusters of blooms (up to 8) produced in terminal or higher axillary cymes. Ten (10) capped ovary stamens that are intermittent bloomers that bloom all year long, but mostly in late winter or early spring; five (5) recurved white or fading cream petals; and five (5) green stamens. Fruit is oval, shiny, red, and measures 1 cm in length. Its seeds are embedded in a watery, bitter flesh. Depending on the weather, there are one or two seeds per fruit. The seeds are spherical or occasionally flattened on one side (Little and Woodbury 1974).

# **Anatomy**

#### Leaf

The cuticle layer that covers the epidermal region was present in the current investigation. The adaxial epidermal cells are larger than the abaxial epidermal cells. All however, dicotyledons. possess characteristic. During leaf transection, mesophyll was able to discriminate between a well-formed palisade and the surrounding spongy layer. The anatomical slices showed a number of palisade layers as well as larger, closer-together palisade cells. Transverse section of leaves revealed biseriate palisade layers. Since only vascular bundles with an arc form were seen. The literature claims that environmental factors affect the cuticle's thickness and existence. Therefore. ecologically relevant it is more than taxonomically (Figure 4). In the previous literature we have studies that the Wilkinson enumerates several environmental factors that affect cuticle thickness and notes that cuticle thickness is generally effective as a climatic or habitat indicator. It has been hypothesized that in vivo leaves would have cuticles but in vitro leaves would not because in vivo leaves are exposed to the sun. Blossoms, the latter also had protruding stigmas, and the former's blooms were smaller in size. Based on the morphological and anatomical features of

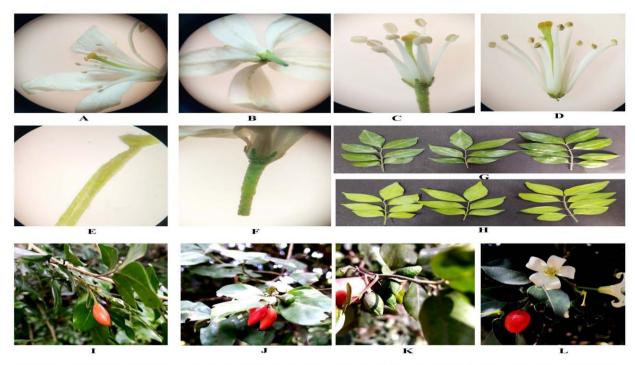


Figure 3. Murraya paniculata (L.) Jack showing Flower under microscope[A (Front view), B (Ventral view)]; (C, D) Stamens; (E)Gynoccium; (F) Sepals with Trichomes; (G, H) dorsal and ventral sides of leaflets with different number of leaves; (I-L) Variation in the Fruit color, shape and size

their leaf lamina, there were not many differences between the Murrava paniculata species that were studied in vitro and those that were studied in vivo. Epidermis: The morphological properties of leaves developed in vitro and in vivo vary, ranging from isodiametric to uneven in their adaxial and abaxial epidermal cells. The adaxial epidermal cells are larger than the abaxial epidermal cells. This characteristic, however, is not taxonomically significant because it is shared by all dicotyledons. Mesophyll: During leaf transection. mesophyll was able to tell the difference between a well-formed palisade and the surrounding spongy layer. Several studies on the ecological anatomy of tree species (Hanson 1917; Ryder 1954) demonstrate that the degree of mesophyll differentiation is highly dependent on the quantity of light exposure. They assert that compared to leaves growing in the shade, leaves grown in the sun have larger, closertogether palisade cells and more palisade layers. Palisade cells in leaves that receive a lot of shade usually grow elongated, thin, disorganized. The biseriate palisade layers observed in in vivo leaves may be the result of this. Bundles of arteries: Since only arc-shaped vascular bundles were seen, the vascular bundles in both in vitro and in vivo leaves are consistent (Taha and Noorma 2008).

### Stem

The epidermis displayed the uniseriate, unicellular trichomes. Phloem and xylem cells were also evident in the slice. Cutting a portion of the stem revealed many uniseriate, unicellular trichomes on the outer layer of the epidermis and oil glands beneath the epidermal layer. Furthermore, the epidermis was encircled by the surrounding cuticle. Numerous dense layers of parenchymatous and polygonal cells made up the cortex. There were open, collateral, and conjoint vascular bundles visible. In the study of the plant's stem anatomy, the oil glands, vascular bundles, and epidermis were recorded. The plant's stem has vertical vascular bundle portions and small hairs on the outside. In the previous reports not even, a single valid report found for the anatomy of stem. So, it is the first report for the anatomy of stem of M. paniculata (Figure 4).

## **CONCLUSION**

For the first time, a thorough comparative study was conducted on *M. paniculata* germplasm that was gathered from different altitude ranges in the Himachal Pradesh Himalayas. According to the current study, *M. paniculata* is widely distributed and prevalent in Himachal Pradesh. There may be reports of its

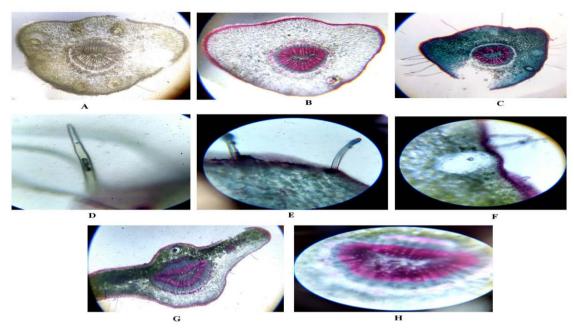


Figure 04. Section cutting of stem (A, B, C); Trichomes on the stem epidermis (D, E); presence of Vacuole in section cutting (F); section cutting of leaf (G, H) of Murraya paniculata (L.)

different other morphotypes with maximum biomass from different places. The availability of the new ecotypes and chemotypes is incredibly likely. Thus, in order to facilitate future research, comparative studies pertaining to phytochemistry, ecological differences, and

bioactivities need to be connected to recently described morphotypes. In order to appropriately employ new morphotypes or ecotypes for a variety of industrial uses and improve the standard of living for the people living in the Himalayan state.

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