Morphological traits, habitat diversity and genetic framework of *Bauhinia* genus

Manish Dev Sharma^{*1}, Tanisha Debnath², Anshu Kumar², Vijaylaxmi Sah², Sonika Kalia²and Indra Rautela²

1-Department of Biotechnology, School of Basic and Applied Sciences, Shri Guru Ram Rai University, Dehradun- 248001, Uttarakhand, INDIA

2- Department of Biotechnology, School of Applied and Life Sciences, Uttaranchal University, Prem Nagar-248001, Dehradun, Uttarakhand, INDIA

*Corresponding Author email- sharma.manishdev@gmail.com

Abstract

The Bauhinia genus, belonging to the family Leguminosae (subfamily Caesalpinioideae), is the largest group under the tribe Cercideae, with approximately 300 species. These species have a variety of morphological characteristics, such as crescent-shaped hilum, bilobed funiculus, bifoliate leaves, and zygomorphic flowers, and they grow as trees, shrubs, lianas, and herbs. Despite its importance, detailed studies on *Bauhinia* as a single issue are scarce, frequently eclipsed by classifications of its extremely polymorphic members. The genus, known by numerous names around the world-including "Orchid Tree" in English and "Kachnar" in Hindi-has significant ecological, nutritional, and therapeutic importance, as recorded in both traditional Ayurveda and modern studies. Bauhinia species are found in North America, the Asian subcontinent, and tropical climates worldwide. Bauhinia species are native to the Himalayan areas of India. Bauhinia species, which are native to India's Himalayan areas, flourish in a variety of soil types, from clayey to sandy, and are resistant to waterlogging and moderate saline levels. The genus contains important species such as B. variegata, B. purpurea, and B. vahlii, each with distinct morphological and ecological characteristics. Advances in genomic profiling have revealed evolutionary pathways within Bauhinia, as well as links with allied genera like Cercis and different genomic markers. Tools and bioinformatics software such as MUSCLE, IQTree, and BUSCO have made thorough molecular analysis possible, revealing genetic diversity and phylogenetic links.

Key words: Bauhinia genus, morphological characteristics and genetic studies

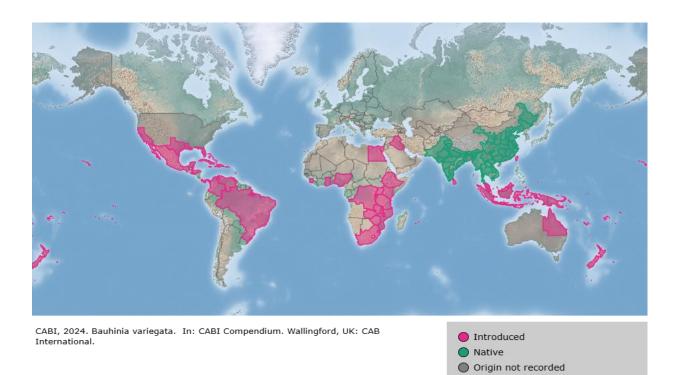
Introduction- The *Bauhinia* genus is the largest in the tribe *Cercideae*. It is characterized into varieties of species that are morphologically distinguished by seeds with crescent shaped hilum and aril-lobed funiculus, simple (entire to bilobed) or bifoliate leaves and zygomorphic flowers. Other

variations are its classifications as trees, herbs, shrubs, or lianas and habit types. A smaller number of authors have studied the *Bauhinia* genus as one topic, instead of focusing on their classifications on the highly polymorphic group (Sinou et al., 2009). Research papers as well as India's traditional *Ayurveda* have documented the potential of Bauhinia genus in the field of medicine and nutrition. Bauhinia genus includes over 300 species and belongs to the family *Leguminosae* (*Caesalpinioideae*). The plants belonging to this genus are commonly known as 'cow's paw' or 'cow's hoof' due to the structure of their leaves (Awasthi & Verma, 2020) (Filho, 2009)

Bauhinia is known to people in different names in different languages-

English- Orchid tree, Mountain ebony.

Hindi- Kachnar Sanskrit: Kachnar Chinese: Yáng Tí Jiă



Geographical Distribution- The trees of *Bauhinia* genus are widely found in North America and the Asian subcontinent. Many species of *Bauhinia* have been reported to grow in India on its Himalayan region from sub-Himalayan tract and outer Himalayas of Punjab and Sikkim, at the

altitude ranging from 650m to approx. 1300 m (Awasthi & Verma, 2020). Bauhinia variegata originated in temperate and tropical China, India, Bhutan, Nepal & Pakistan and South Eastern Asia. It was introduced, adapted and cultivated in Africa, tropical America, West Indies, and on several islands of India & Pacific Ocean (Kamal et al., 2022) (Flora of China Editorial Committee, 2014). It grows on both heavy clayey and light sandy soils with neutral reaction. The best growth is observed on medium-texture deep loamy soil, prefers well drained soils. It can withstand seasonal waterlogging and moderate saline soil (Carlowitz, 1991). Bauhinia vahlii Wight and Arn. Is a multipurpose woody climber, of 10m to 30m long height and 20cm of girth widely located in India, Bhutan, Mauritius, Pakistan, Sri Lanka and Africa (Thakur et al., 2022)

Bauhinia purpurea (purple bauhinia) is small tree with relatively large leaves (upto 20cm wide). Native to Southern and South-Eastern Asia. Introduced to Taiwan, Mexico, Egypt. Usually grows in saline soil in tropical and sub tropical regions of the world. A brief data highlight in Table-1 on the basis of its geographic distribution in India, provided by <u>eFlora of India 2020</u> and <u>India biodiversity portal</u>.

S. no	Species	Habit & Forest	Common name	Distribution
1	Bauhinia acuminata	Shrub-Dry deciduous forest	Dwarf white orchid tree	Western Ghats and Eastern ghats
2	Bauhinia malabarica	Tree-Dry deciduous forest	Malabar Bauhinia	Western Ghats and Eastern ghats
3	Bauhinia purpurea	Moderate sized tree- Dry deciduous forest	Purple Orchid tree	Western Ghats and Eastern ghats
4	Bauhinia racemosa	Small tree- Dry evergreen to Dry deciduous forest	Bidi leaf tree	Throughout India excluding North-eastern & Northern region
5	Bauhinia tomentosa L.	Shrub-Woodland Forest found in plains to low Altitude	Yellow orchid tree	Western Ghats
6	Bauhinia vahlii Wight & Arn.	Climber- Dry deciduous forest	Maloo creeper	Western Ghats
7	Bauhinia variegata L.	Moderate sized tree- Dry evergreen to Dry deciduous forest	White, purple orchid tree	Western Himalayas & North-East India

Table 1 In list the habitat and distribution sites of different *Bauhinia* species

Morphological Characteristics-These images are sent from the Tripura Forest Department, Sepahijala branch. The morphological characteristics of various species of *Bauhinia* genus will aid in determining their identity (<u>Makwana et al., 2014</u>). As these serve as medicinal extracts for several diseases, the distinguishment might help as a strong diagnostic "Comparative Morpho-micrometric Analysis of Some Bauhinia Species (Leguminosae) From East Coast Region of Odisha, India," 2020a). Here, the listed below Table-2 characterizes the leaf morphology, extracted from different locations. The measurements & frequency may slightly vary from region to region in each case.

S.no	Leaf Features	B. variegata	B. racemosa	B. tomentosa	B. vahlii	B. blakeana	B. malabarica	B. purpurea
1.	External	Pulvinous bifolate	Pulvinous bifolate	Pulvinous bifolate	Pulvinous bifolate	Pulvinous bifolate	Pulvinous bifolate	Pulvinous bifolate
2.	Margin	Cleft,lobed	Emarginat e	Emarginate	Deeply lobed emarginate	Entire	Entire (not lobed or toothed)	Entire (not lobed or toothed
3.	Size	~10.2x4 cm ² - 16.4x8 cm ²	\sim 7x9 cm ²	~6x10 cm ²	\sim 12x45 cm ²	~10x13 cm ²	~3.5x4.5 cm ²	~11x14.78 cm ²
4.	Stomata type	Anisocytic,An omocytic, paracytic	Anisocyti c,Anomoc ytic	Paracytic	Anomocytic	Anisocytic, Paracytic	Anisocytic	Anisocytic, Paracytic
5.	Stomata frequency	Varies	14- 20/mm ²	13- 76.66/mm ²	288.33/mm ²	24/mm ²	30/mm ²	18//mm ²
6.	Veination pattern	Reticulate	Reticulate (Actinodr amou)	Reticulate (Actinodra mou)	Palmate Reticulate	Campylodro mous	Actinodramous	Campylodrom ous

 Table 2 Morphological comparation between the various Bauhinia species

Molecular Profiling- Recent studies on molecular level of *Bauhinia* genus have revealed more information on their characteristics. Table-3 Listed the brief on genomic profiling of *Bauhinia* genus including the software used and their functions.



Figure 1: flower of Bauhinia variegata





Figure 2: flower of Bauhinia

Figure 3: leaf of Bauhinia variegata

Table 3 Software analysis to aligned the genomic profiling of Bauhinia genus

Samples	Outcome	Software used	Functions of software	Reference
Bauhinia variegata L Bahamas, USA,		Muscle 3.6	Used to sequence alignment under default settings	(<u>Sinou et al.,</u> 2009)

(Florida,	absence and	Bioedit 7.0.8.0	Used for verifying the	
cultivated), Mexico.	presence of this	<u>Diocult 7.0.0.0</u>	alignment of sequences	
Bauhinia purpurea	region was not		angliment of sequences	
LSingapore	explicitly			
Botanical Garden,	mentioned for all			
Australia.	species of	IndelCoder	Used for coding gaps as	
Bauhinia tomentosa	Bauhinia genus.	mucreouci	separate characters using	
LKirstenbosch	0		both the modified complex	
Botanical Garden,			indel coding (MCIC) and	
USA(Hawaii)			simple indel coding (SIC)	
			algorithm.	
		SeqState version 1.25	Used for coding gaps with	
		<u> </u>	SIC algorithm for the	
			Bayesian Analysis	
		MrBayes	Bayesian analysis software	
			used	
		Sequencher 4.7	Software used for	
		<u> </u>	sequencing assembly and	
			editing.	
		BigDye Chemistry(version1.1)	Applied for sequencing	
			reactions.	
		BioEdit	A user-friendly biological	
			sequence alignment editor	
			and analysis program.	
Leaf tissues of	The complete	IQTree	To build maximum likelihood	(<u>Gu et al.,</u>
Bauhinia variegata L.	plastome is analysed		tree for phylogenetic analysis.	<u>2020</u>)
<i>var. variegata</i> from South China	to be 155,415 bp in length and exhibits	ModelFinder	Models recommended by it	
South China	the typical		were used for the	
	quadripartite		phylogenetic analysis based on a data matrix of a	
	structure, including		concatenation of TTCDS	
	two inverted repeat		genes.	
	(IR) region of	GetOrganelle pipeline	Used for assembling the	
	86,110 bp and a small single copy		plastome from clean	
	(SSC) region of		sequencing reads	
	18,207 bp.	Geneious	Used to verify the accuracy of	
	Strong clade		the assembly and annotating the plastome	
	relationship	Plastid Genome Annotator (PGA)	Used forn annotating the	
	between B.variegata var.		plastome	
	B.variegata var. variegata & B.	MAAFT	Used for aligning the data	
	blakeana; sister to		matrix during the	
	<i>B. acuminata</i> L.		phylogenetic analysis	
	with a strong			
	bootstrap value.			
12 samples belonging	Molecular profiling	FreeTree program	Used to estimate genetic distances based on Jaccard's	(<u>MATHEW,</u> 2010)
to Bauhinia genus	of <i>Bauhinia</i> accessions using		similarity coefficient and for	<u>2010</u>)
	RAPD markers		cluster analysis based on	
			genetic distance using the	
			Neighbour Joining Program	
			(NJ).	
		FreeTree	Employed for a 500 replicate	
			bootstrap analysis and to	
			generate a NJ tree based on	

			the distance data obtained from the RAPD band data analysis.	
Sample of Bauhinia variegata	Evolutionary trajectory within	Benchmarking Universal Single Copy Orthologs (BUSCO)	Used for genome quality evaluation	(<u>Zhong et al.,</u> <u>2022</u>)
DNA extracted from leaves- whole genome sequencing	genus as close relationship has been observed with	Repeat Masker & Repeat Modeler	Repeat sequence identification	
RNA from flower, fruit, roots and leaves-	<i>Cercis</i> and distinct branch length after	HISAT2	Used for RNA sequence data mapping	
transcriptome sequencing	divergence from C. canadensis	SAMtools	Used for data merging	
		StringTie	For transcript extraction	
		<u>TransDecoder</u>	For coding region prediction	
		Efficiently Dealing with Transposable Elements Using Assembly (EDTA)	Tool or pipeline used for identifying repetitive sequence, particularly transposable elements, in genomic data.	
		<u>GETA v2.4.5</u>	Used for protein coding gene prediction	
		Genewise	For homology-based prediction	
		Augustus	For predicting protein coding genes with the incorporation of user defined constraints.	
Fresh young leaves of <i>Bauhinia varieagta</i> from an individual	The mitogenome of Bauhinia variegata	Trimmomatic v0.39	Used for filtering illumina reads with default parameters during data	(<u>Sun et al.,</u> <u>2024</u>)
location of Guangdong,China	covered 1.46% of	Bandage	For visualizing the original	
Guanguong,ennia	the genome		assemblies.	
	Contained 36		Choosing mitochondrial	
	protein-coding		contigs based on depth and	
	genes, 17 tRNAs 3		sequences similarity.	
	rRNAs Relationship with <i>Tylosema</i> <i>esculentum</i> within subfamily <i>Cercidodeae</i>		Exporting the mitogenome sequence of <i>Bauhinia variegata</i> .	

This study has shown phylogenetic and biogeographic history of the genus *Bauhinia* sensu lato and related genera in tribe *Cercidae*. The plastid trnL–trnF region of 85 species, which collectively are typical of the eight genera classified within *Bauhinia* s.I., have had their plastids sequenced as part of their primary effort to better understand the taxon connections within *Bauhinia* and between it and allied genera. The region trnL-trnF refers to the specific region of chloroplast genome of plants that includes the trnL gene coding for leucine, and trnF gene coding for Phenylalanine.

In <u>MATHEW (2010)</u>, the study involved the molecular profiling of *Bauhinia* accessions using RAPD markers. The DNA was isolated from 12 plant samples of *Bauhinia* genus following Dellaporta (1983) protocol to assess the genetic variability within and among *Bauhinia* accessions. This study aimed to identify polymorphic banding patterns, determine pairwise distances among Bauhinias and generate a NJ tree to understand the genetic relationship between the samples. In Zhong et al. (2022), the aim was to test the hypothesis of the allotetraploid origin of the plant species by conducting a comprehensive analysis of its chromosomal level genomic assembly of *Bauhinia variegata*. In Sun et al. (2024), their aim was to assemble and characterize complete mitogenome of *Bauhinia variegata* to provide genetic information for phylogenetic studies within Leguminosae family.

Secondary Metabolites & Benefits-*Bauhinia* genus is known for consisting diverse array of secondary metabolites, which has been studied over the years. Mainly, three types of metabolites are found and studied widely namely, Flavonoids, Terpenes, Quinones. Flavonoids are the main compounds that are responsible to exhibit hypoglycemic, antioxidant and anti-Cancer activities and these are one of the Quionones. (Kaewamatawong & Ubon Rajathanee University, 2551) (Filho, 2009)

In the study <u>Kamal et al. (2022b)</u>, specific flavonoids were identified in different species of Bauhinia genus. These includes Kampferol, Quercitin and derivatives of flavones and flavonols. *Bauhinia megalandra* is found to have Quercitin, a flavonol known for its antioxidant and anti-inflammatory properties. (<u>Kaewamatawong & Ubon Rajathanee University, 2551</u>). *Bauhinia longifolia* in <u>Aquino et al. (2019)</u> has reported myricitrin, juglanin,afzelin and bauhiniastatin 2 for the first time. Bauhiniastatin 2 has anti-Cancer properties.

<u>Filho (2009)</u> includes other cases of flavonoids such as *Bauhinia forficata* consists of flavonoids responsible for antioxidant activity and hypoglycemic activity. *Bauhinia racemosa* has flavonoids functional for antipyretic activity. *Bauhinia variegata*, the mostly studied species of Bauhinia genus has flavonoids functional for preventing tumour.

Terpenes can be detected through phytochemical analysis. Essential oils are extracted from Bauhinia species, and they are observed to contain varieties of terpene secondary metabolites. The terpenes are responsible for biological activities like antioxidant properties, Anti-inflammatory effects, Anti-cancer as well as antidiabetic effects and anti-microbial properties. (Filho, 2009) (Pichika et al.,

<u>2010</u>) (Ahmed et al., 2012). These properties play a major role in pharmaceutical and nutraceutical applications. Few compounds of terpenes found in Bauhinia genus are Sesquiterpenes such as β -caryophylle, α -copaene, δ -Cadinene, Elemol, Germacrene-D-4-ol, Globulol and Oxygenated Sesquiterpenes such as 2,3-Dihydrofarnesol, 8- α -11-elemendiol farsenol, (Z,E)-Farsenol, (Z,Z)-Farsenol, Guaiol. (Aquino et al., 2019)

Rhizosphere Environment- *Bauhinia petersiana* is a leguminous dicotyledonous plant, that is found to grow in the dry Agro-ecologies of South Africa. Morphologically, *Bauhinia petersiana* does not have any root nodules, thus they depend on soil nitrogen. Through experimental analysis of rhizosphere soil, collected from *Bauhinia petersiana* plants in East and West Kavango regions of North Eastern Nambia, *Azospirillum* genus was isolated and identified. (Haiyambo & Percy, 2018)

A diverse community of arbuscular mycorrhizal fungi (AMF) sustains symbiotic relationship with *Bauhinia faberi var*. microphylla, proven by molecular techniques. The root system of *Bauhinia faberi var*. microphylla affects the shaping of rhizosphere microbial communities. The Community Composition of Arbuscular Mycorrhizal Fungi in the Rhizosphere of *Bauhinia* Faberi Var. Microphylla in the Dry Valley of Minjiang River. The rhizosphere of *Bauhinia* genus contributes to nutrient cycling i.e., nitrogen and phosphorus cycles with the associated microbiome helping mobilize these nutrients in the soil. (Peccatti et al., 2020)

Conclusion- The species numbering over 300, belonging to *Bauhinia* genus are widely spread and located globally. Their native, introduced & cultivated regions vary for each species(India biodiversity portal). *Bauhinia* genus has potential pharmaceutical and nutraceutical applications. *Bauhinia vahlii* Wight and Arn. has anti-inflammatory, antioxidant (Sowndhararajan et al., 2010) (Sowndhararajan & Kang, 2013), antimicrobial properties "Comparative Morpho-micrometric Analysis of Some *Bauhinia species* (Leguminosae) From East Coast Region of Odisha, India," (Pichika et al., 2010b). *Bauhinia longifolia* has flavonoids that shows the highest antiviral activity (SCIELO - Scientific Electronic Library Online, n.d.). Some species even have anti-cancer properties that can bring revolution in cancer medicine formulations. Apart from the potentials in these fields, medicines made of several species of *Bauhinia* genus are being used in Ayurveda, Folk medicines of many regions and Traditional Chinese medicine. These species are categorised under "Least Concern" by the IUCN Red List Category and the most popularly studied species is *Bauhinia variegata*. *Bauhinia variegata* has shown antioxidant, anti-cancer and anti-microbial activities

(Mishra et al., 2013) and it scavenged both the superoxide radical anion and the beginning stage of lipid peroxidation (Mesquita et al., 2022). These biological activities show possibilities for finding breakthrough in antihyperglycemic therapies, which makes it possible to create medications with targeted benefits and fewer side effects. The studies in the last decade revolving around the genomic profiling of several species of Bauhinia genus has unfolded uncountable aspects of new formulations. The drawback is that, several plants species belonging to this genus are yet to be studied. The whole data is not known and available to us yet. Wrong determination of species and commercial availability of them may result in variable outcomes in experimental setups (Sinou et al., 2009). Considering the positive points, more research and experiments are expected in the upcoming years on this topic.

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