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# Genetic status of *Centella asiatica* (L.) Urb. (Indian pennywort): A review

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## ABSTRACT

In recent years, demand for medicinal plants increased due to the rise in attraction towards herbal products which are safer compared to modern drugs. *Centella asiatica* (L.) Urb is known as an important medicinal plant in herbal medicinal systems. It is also used as an active ingredient for many products in the pharmaceutical and cosmetic industry. So far, review on this plant concerns mainly on medicinal, cosmetology and photochemical works reported. This review presents the genetic studies conducted on this herb along with a mention of conservation. Since documenting and studying genetic variation and its composition has an important connection for the understanding of evolution and improving the conservation of this species.

**KEYWORDS:** Genetic diversity, medicinal plant, genetic marker, herbal medicine, conservation

## INTRODUCTION

Apiaceae family is generally well-known for vegetables, medicinal and flavoring plants. *Centella asiatica* (L.) Urb, comes under this flowering family which is an important medicinal and vegetable herb that has a significant position in the trading market. Mostly secondary metabolites in plants are used as food additives, drugs, pesticides and pigments. Generally, molecular markers based methods are essential to study diversity, classify species, identifying adulteration, predict gene flow and track the trait loci in the plant for breeding and genetic improvement (Neelima, 2017).

Many medicinal plant species we use commonly are mostly harvested from the wild but its conservation, improvement in cultivation and breeding are not targeted when compared to the importance given to crop plants. *C. asiatica* is one such medicinal herb that requires proper wise utilization, conservation and improvement at the genetic level. This moisture loving prostrate herb widely utilized in diverse fields such as pharmaceutical, dermatological, cosmetic, traditional and ayurvedic medicinal preparations is largely considered as brain food and panacea (Ravi *et al.*, 2019).

For industry and commercial purposes, *C. asiatica* is mostly collected from the wild and natural resources; hence to ensure sustainable utilization it is necessary to have organized commercial cultivation and also its biomass and biosynthesis

of compounds influenced by genotype (G) x environmental (E) are to be evaluated to make improvements in this species. The above mentioned require initially proper documentation and understanding of this species genetic variation. This review gives a summary of genetic and genomic studies in *C. asiatica* to help in genetic improvement and conservation.

## DISTRIBUTION, DEMAND, USES

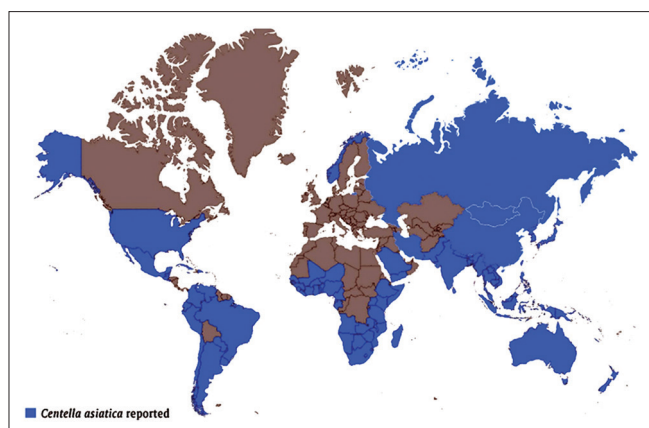
*Centella asiatica* (L.) Urb. (Figure 1), also generally known as Gotu kola, Asiatic pennywort, Indian pennywort, Tiger herb or Spade leaf. It is a perennial herb, prostrate, faintly aromatic, stoloniferous and creeping runner. It is diploid with  $2n = 2x = 18$ , but some studies reported the presence of B chromosomes (Das & Mallick, 1991) and tetraploid (Kokubugata *et al.*, 1998). Reproduce is through seeds and vegetation propagation. It is distributed in the regions of Southeast Asia (Cambodia, Indonesia, Malaysia, Vietnam, Thailand, Singapore, the Philippines, Myanmar, Laos, Christmas Island and the Andaman Islands), Oceania, Africa, America, China, India, Sri Lanka, Pakistan, Madagascar, South East USA, Mexico, Pacific Islands, Australia, Venezuela, Columbia, Nepal etc (CABI, 2020) (Figure 2).

Over three thousand years ago, *C. asiatica* is being used as a “panacea” in India, China, Philippines, Africa, Madagascar and Sri Lanka. In the world, Madagascar is the top *C. asiatica* producer. In Southeast Asia, it has been used in the treatment of

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**Figure 1:** Habit of *Centella asiatica* at JNTBGR experimental field



**Figure 2:** Distribution map of *Centella asiatica* reported

many problems such as rheumatism, skin diseases, inflammation, mental illness, syphilis, diarrhoea, epilepsy, dehydration and hysteria. In Europe, it was utilized in the treatment of wounds and ulcers. In traditional Asian medicine, *C. asiatica* has been used for hundreds of years, chiefly in dermatological conditions to get better from burns, small wounds, hypertrophic wounds and scratches. It is also suggested as an anti-inflammatory agent, mostly in eczema, in the treatment of vein insufficiency, antipyretic, anti-cancer agent, diuretic, an antibacterial, rheumatic, antiviral drug, for improving cognition and useful in relieving anxiety (Sudhakaran, 2017).

The efficacy of *C. asiatica* is due to the presence of pentacyclic ursane type triterpenoid centellosides such as madecassoside, madecassic acid, asiaticoside and asiatic acid. Due to the presence of immense medicinal properties, this plant was incorporated into many Pharmacopoeias. In the 19<sup>th</sup> century, *C. asiatica* was included in the Indian Pharmacopoeia and many others included viz. European Pharmacopoeia VI, Chinese Pharmacopoeia, Homöopathisches Arzneibuch, Dutch Pharmacopoeia, British Herbal Pharmacopoeia, La Farmacopea Italiana X, German Pharmacopoeia, Polish Pharmacopoeian Martindale European Pharmacopoeia and Pharmacopoeie française (Bylka *et al.*, 2013). It has great demand in the trade

market, according to the United States market status *C. asiatica* comes in the list of top 25 selling medicinal plants and comes under the category of one of the top eight best selling herbs in the USA and Europe (Rohini *et al.*, 2019). According to the Indian Medicinal Plants Database, all plant parts of *C. asiatica* (Sanskrit name: Mandukaparni Brahmi) (National Medicinal Plants Board, 2015) as Brahmi booti in India and it was listed as a highly traded herb (estimated annual trade 500–1000 MT; sale at the rate of 30–35 Rupees per Kg).

## GENETIC STUDIES EXPLORED THE *C. ASIATICA* GENOME

During the past years, many genetic works were conducted on this species to know about its genetic structure. The researchers used various genetic markers (Table 1) to study the *C. asiatica* genome and many genes were characterized. Recently, next-generation sequencing technology was used to explore genome size. In this status, the present review provides an overview of the genetic works in *C. asiatica*.

Genetic mapping can be used as a first step towards the improvement and efficient breeding of plants including medicinal plants where genetic markers play a key role. The difference among individual plants generally lies in the “genetic material”, i.e. DNA (deoxyribonucleic acid). The specific region in the chromosomes is responsible for specific characters which are termed genes. After the discovery of sequencing, it was easy to identify those character responsible regions in chromosomes. But still, several researchers are using the indirect method for identifying those regions called genetic markers (Agarwal *et al.*, 2008; Semagn *et al.*, 2006; Sharma *et al.*, 2018).

Genetic markers are not a recent discovery since it was used during the nineteenth century by Gregor Mendel. He used phenotype-based genetic markers in his experiment. Genetic markers can be used to distinguish individuals by representing the genetic differences in the genome. After the discovery of polymerase chain reaction (PCR) by Kary Banks Mullis in 1983, several marker systems arise to play a key role in molecular research. PCR based molecular markers reduce the effort, expense and time required for molecular mapping. Now a day's SSR and SNP-based GMMs (genic molecular markers) are the most preferred markers for studying genetics and breeding plants even though other various markers like ISSR, RAPD, and AFLP etc., are easily available (Al-Samarai & Al-Kazaz, 2015; H. Chen *et al.*, 2017; Collard *et al.*, 2005; P. K. Gupta *et al.*, 1999; Joshi *et al.*, 1999; P. Kumar *et al.*, 2009; Semagn *et al.*, 2006; Sharma *et al.*, 2018; Srivastava *et al.*, 2009).

## Randomly Amplified Polymorphic DNA (RAPD)

RAPD was used to assess genetic variation in populations and to find the phylogenetic relationship among species/subspecies and cultivars and to the construction of genetic linkage map (P. K. Gupta *et al.*, 1999; P. Kumar *et al.*, 2009; Welsh & McClelland, 1990; Williams *et al.*, 1990). *C. asiatica* plants have been mostly explored by these markers initially.

**Table 1: Summary of genetic studies in *Centella asiatica* using molecular markers**

Type of Marker	Samples/ Accessions/ Populations (Location)	Number of Primers	Genetic parameters or indices and Comments	References
1.RAPD 1.1	21 Ecotypes (Nepal)	Out of 21 decamers, 8 were used for analysis	81% polymorphism showed by 8 primers, average 4.37 polymorphic bands per primer, 0.52–0.911 Jaccard similarity coefficient; RAPD PCR condition was standardized	(Devkota, 2011; Devkota <i>et al.</i> , 2011)
1.2	10 Accessions (Kerala, Tamil Nadu, India)	Out of 40 decamers, 4 used for analysis	Average 5 bands per primer, 0.71–0.88 Nei & Li's coefficient of similarity; Nagercoil (Tamil Nadu, India) and Ponnudi accessions shared 0.88 similarity coefficient, Pattami accession (Palakad, India) showed distinct cluster	(Krishnan <i>et al.</i> , 2007)
1.3	18 Medicinal plant species include <i>C. asiatica</i> (Karnataka, India)	Out of 10 primers, 3 primers used for analysis	96.84% polymorphism showed by 3 decamer primers, 0.00–0.72 Pairwise Nei & Li's coefficient of similarity; Analysis differentiated medicinal plants species into groups (Chikkaswamy, 2015); Clusters indicated low gene in different medicinal plant species (Radhika <i>et al.</i> , 2012) .	(Radhika <i>et al.</i> , 2012) (Chikkaswamy, 2015)
1.4	18 Medicinal plant species include <i>C. asiatica</i> (Karnataka, India)	13 primers	97.6% polymorphism; Analyzed species showed high genetic differentiation coefficients (Gst)	(Paramanik & Chikkaswamy, 2014)
1.5	Nine accession (Andra Pradesh, India)	Out of 30 primers, 16 were used for analysis	87% polymorphism, 8.5 average no. of bands per primers, Mean genetic diversity 2.6; Cluster result indicated no correlation between genetic distance and geographical distances (Distant accessions showed 78% similarity)	(Padmalatha & Prasad, 2008)
1.6	Nine regenerated plants (Lucknow, India)	Out of 40 primers, 19 RAPD were selected	Average 3.15 bands per primer as average; <i>C. asiatica</i> auxiliary buds/nodal encapsulates efficiently stored up to 200 days; Confirmed genetic stability of the regenerated plants form artificial synthetic seed	(Archana Prasad <i>et al.</i> , 2014)
1.7	13 accessions of <i>C. asiatica</i> (South India) and <i>Hydrocotyle</i>	15 RAPD markers out of 25	Mean Nei gene diversity 0.2627, 99.18 % polymorphism; Cluster analysis produced single cluster with four sub clusters for <i>Centella asiatica</i> ; Cluster analysis differentiated <i>Centella asiatica</i> from <i>Hydrocotyle</i>	(Thomas, 2010)
2. AFLP	4 accessions (Bhowali (A), Darjeeling (B), Bangalore (M), Assam (E), India)	23 primers combinations	80.9% polymorphism, 0.04–0.38 Jaccard similarity coefficient; The accessions M (7.38g centellosesides/m <sup>2</sup> ) and A (total centellosesides yield per unit area 5.25 g/m <sup>2</sup> ) suggested for commercial cultivation under agro climatic conditions of the northern Indian plains	(A Prasad <i>et al.</i> , 2014)
3. EST-SSR and RAPD 3.1	10 plants species include <i>C. asiatica</i> (Bangalore, India)	10 EST-SSR; 4 RAPD primer	UPGMA (RAPD) cluster analysis separated <i>C. asiatica</i> as one cluster and phylogenetic analysis (SSR) clustered <i>C. asiatica</i> with <i>Anethum graveolens</i> , <i>Coriandrum sativum</i> species; Markers suggested as capable of transferring to closely related species belonging to Apiaceae and Brassicaceae.	(Neelima, 2017)
4. ISSR 4.1	162 individuals (14 populations) (China)	Out of 100 ISSR, 10 selected for analysis	Average 9.8 bands per primer; Species level (Nei genetic diversity (H) = 0.2183, percentage of polymorphic loci (P) = 66.33%), and population level (H ranged from 0.021–0.1610, P = 5.1–43.88%), Low gene flow = 0.2607; Suggested conservation of high genetic diversity population viz. Jinhua, Fuzhou and Jiujiang populations for good agricultural practices.	(X. G. Zhang <i>et al.</i> , 2012)
4.2	<i>C. asiatica</i> and <i>Hydrocotyle verticillata</i> (Indonesia)	30 ISSR	78% polymorphism; Four markers potentially distinguish <i>H. verticillata</i> and <i>C. asiatica</i> .	(Subositi <i>et al.</i> , 2016)
4.3	40 individuals (from 3 Species) (Australia)	Out of 34 primers, 14 were selected for analysis	87.8% polymorphism, 0.47–1.00 (Nei's similarity coefficient), <i>C. asiatica</i> and <i>C. cordifolia</i> were clustered at similarity of 0.61 and were hereditarily closer, but divergent from <i>C. erecta</i>	(Alqahtani <i>et al.</i> , 2017)
5. Genomic SSR 5.1	17 Accessions (16 Madagascar, South Africa and 1 India)	Out of 30 SSR, 20 were selected for analysis	Average 4.3 bands per primer, mean observed heterozygosity 0.114, mean expected heterozygosity 0.379; Genomic microsatellites markers developed and characterized.	(Rakotondralambo <i>et al.</i> , 2012)
5.2	32 accessions (Madagascar, India, South Africa)	12 SSR; 5 pairs of universal chloroplastic primers	2.09 (Diploid) and 9.5 (Tetraploid) alleles per locus, expected heterozygosity (Diploid) = 0.133–0.668; 0.52 Mean PIC value; SSR analysis distinct genotypes Indian and South African. Two chlorotypes (diploid and tetraploid populations) disclosed by chloroplast analysis.	(Rakotondralambo <i>et al.</i> , 2013)
5.3	17 genotypes including varieties (India)	20 Genomic SSR	Average 3.1 alleles per locus, 92.96% polymorphism, expected heterozygosity 0.456, PIC 0.390; DNA barcode for 17 genotypes developed using 10 SSR markers	(Rohini MR, Anuradha Sane, Ravish Chaudhary, 2019)

(Contd...)

Table 1: (Continued)

Type of Marker	Samples/ Accessions/ Populations (Location)	Number of Primers	Genetic parameters or indices and Comments	References
6. EST-SSR	10 local cultivars (Apiaceae family include <i>C. asiatica</i> )	Out of 18 primers, 6 were amplified effectively	Average 3 alleles per primer, average 0.95 PIC value; Insilico (Sahu <i>et al.</i> , 2015) based EST-SSR markers were developed; <i>Centella javanica</i> showed highest transferability of 83.3% with <i>Centella asiatica</i> .	
6.1	Assam, India			
6.2	30 Accessions (Tamil Nadu, Kerala, India)	10 EST-SSR	Average 1.2 alleles per primer, average PIC value 0.0195; The molecular screening through microsatellite markers showed low polymorphism	(Sakthipriya <i>et al.</i> , 2018)

Standardizing the RAPD PCR condition for diversity study is important which was completed using 21 ecotypes by 8 primers in Nepal (Devkota, 2011; Devkota *et al.*, 2011). Krishnan *et al.* studied the variation among the ten ecotypes using 4 decamers in south India (Krishnan *et al.*, 2007). Likewise, *C. asiatica* was analyzed using the RAPD primers which showed differences in banding pattern from other plant species in China (Ruan *et al.*, 2008). Perhaps due to cross-pollination, 87% polymorphism was observed between nine accessions collected from Andhra Pradesh state, India using 16 primers (Padmalatha & Prasad, 2008). These studies confirm the genetic variation found in the different ecotypes and also provide the marker information to differentiate genuine *C. asiatica* from its unauthenticated samples.

In other studies using RAPD primers were used to analyse 18 medicinal plants species including *C. asiatica* (Chikkaswamy, 2015; Paramanik & Chikkaswamy, 2014; Radhika *et al.*, 2012). Another researcher used a combination of marker systems namely AFLPs and Long Primer Randomly Amplified Polymorphic DNA (LP-RAPDs), to study genetic relationships among 12 accessions of *C. asiatica* which were collected from various regions of Peninsular Malaysia. The study identified an accession (CA03) with a different phenotype and shows a unique DNA profile when amplifying with AFLP and LP-RAPD analyses. The lowest percentage of polymorphism was obtained as 8.15% (AFLPs) and 6.18% (LP-RAPDs) in CA05 (Mun, 2003). Thirteen accessions of *C. asiatica* from Southern India along with *Hydrocotyl* species was used to obtain genetic variability through 15 RAPD markers produced four sub-clusters for a single *C. asiatica* cluster in cluster analysis and differentiated *Hydrocotyl* species from *C. asiatica* (Thomas, 2010).

Another group confirmed the genetic stability of the regenerated plants from artificial synthetic seeds using RAPD (Archana Prasad *et al.*, 2014). In another study, SSR and RAPD primers were used to access a diversity of *C. asiatica*. Authors suggested that the markers used in the study were capable of transferring to closely related species belonging to Apiaceae and Brassicaceae (Neelima, 2017). Reproducible 23 AFLP primers were used to screen germplasm of *C. asiatica* which yielded a sum of 696 fragments within this 563 (80.9%) were found to be polymorphic. Accession M has shown genetic divergence from the other A, B, E accessions. They identified the accessions M and A for commercial cultivation (A Prasad *et al.*, 2014).

### Inter Simple Sequence Repeats (ISSR)

This highly polymorphic PCR based marker, dominant in nature, is distributed all over the genome and is widely used in genetic mapping, genetic diversity and evolutionary biology studies. It has advanced over other PCR markers like AFLP and RAPD (P. Kumar *et al.*, 2009; Y. Zhang *et al.*, 2016; Zietkiewicz *et al.*, 1994). Fourteen *C. asiatica* populations from China comprising 162 individuals were used to estimate genetic diversity by ISSR primers. Out of 100 ISSR primers screened, 10 primers produced 98 clear and reproducible fragments. A high level of genetic diversity was obtained at the species level (Shannon's diversity index (I) = 0.3305, Nei genetic diversity (H) = 0.2183, percentage of polymorphic loci (P) = 66.33%) and population level (H ranged from 0.021–0.1610, P = 5.1–43.88%, I = 0.031–0.2376) respectively. But the gene flow obtained was low (Nm = 0.2607). This may be due to, geographical distance barriers that restrict gene flow that lead to such patterns of genetic structure and also some populations such as WC, HS, GZ, GA, WZ, JLP, and EM shown low Shannon's information index (X. G. Zhang *et al.*, 2012).

Dyah Subositi and his research team analyzed *C. asiatica* and *Hydrocotyle verticillata* diversity via the ISSR marker system. Four markers had shown the potential to distinguish *H. verticillata* and *C. asiatica*. The (GA)<sub>8</sub>CTT and (CT)<sub>8</sub>G markers generated specific fragments for *C. asiatica* and one specific fragment of *Hydrocotyle verticillata* produced using (GT)<sub>6</sub>CC and (AG)<sub>5</sub>T markers. This study shows the significance of the ISSR marker as a potential system to differentiate closely allied species (Subositi *et al.*, 2016). Another study used three species of *Centella* viz. *C. asiatica*, *C. cordifolia* and *C. erecta* from Australia. 14 informative ISSR primers were used to estimate the genetic similarities among samples. The analyzed populations were grouped into three clusters in dendrogram based on Nei's genetic distance. *C. asiatica* and *C. cordifolia* were firstly clustered at a similarity of 0.61 and it shows that *C. asiatica* and *C. cordifolia* were hereditarily closer but divergent from *C. erecta* (0.47) (Alqahtani *et al.*, 2017).

### Microsatellites or Simple Sequence Repeats (SSR)

Microsatellite markers have various uses in the genetic studies of linkage in families, ecological research and linkage disequilibrium studies of populations (Al-Samarai & Al-Kazaz, 2015; Litt & Luty, 1989). In eastern Madagascar *C. asiatica* GA/GT-enriched a genomic library was constructed. Out of 30 SSR markers designed



from this library, twenty were tested for polymorphism among 17 accessions of *C. asiatica* (16 from Madagascar and one from India). In this study, seventeen SSRs were found polymorphic and a total of 73 alleles with an average of 4.3 alleles per locus were obtained (Rakotondralambo *et al.*, 2012). In another study, the genetic diversity analysis was performed in 30 accessions of *C. asiatica* using 10 EST SSR markers. The molecular screening through microsatellite markers showed low polymorphism (0.019) between the samples analyzed (Sakthipriya *et al.*, 2018). Sahu *et al.*, used local cultivars of 10 accessions from a different genus of the Apiaceae family to the check effectiveness of the selected 18 primers out of 603 EST-SSR primers designed. Only six primer pairs were amplified at 7 different loci in *C. asiatica* (average PIC value of 0.95) and further, these markers were tested for cross-transferability in nine other important genera/species of the Apiaceae family. The PCR amplification revealed that all the six markers studied were cross-transferable at least in one of these genera and all the primers were polymorphic. In *Centella javanica* highest transferability of 83.3% was observed (Sahu *et al.*, 2015).

Genetic diversity was analyzed in six wild *C. asiatica* populations collected from Madagascar and two other countries accessions using CpDNA and SSRs. Twelve SSR markers and 5 CpDNA primers were used to analyze the genome among the 32 accessions. In the case of SSR, the allelic number varied from 1–4 per population which may be due to whole-genome duplication. Indian accession was included in this study showed more than two alleles. The SSR analysis confirmed the divergence of leaf morphology of Malagasy *C. asiatica* within a sample collected from a single site due to active sexual reproduction (Rakotondralambo *et al.*, 2013). Another research group analyzed seventeen genotypes of *C. asiatica* from India studied using 20 genomic SSR showed a low level of genetic diversity with an average similarity coefficient (0.85) among them. Accessions IHRCA-5 and IHRCA-6 were found as the most distinct and the DNA barcode of 17 genotypes was developed (Rohini *et al.*, 2019). Several studies using different ecotypes and different marker systems produced varying range of diversity values in *C. asiatica*, but marker selection for the practical application was not yet applied widely since markers within species linked to quantitative characters are seldom investigated.

## GENES CLONED AND CHARACTERIZED IN *C. ASIATICA*

HMGR is the first and rate-limiting enzyme of the MVA pathway (mevalonate pathway) and might play important role in triterpenoid biosynthesis. Molecular cloning, characterization and expression analysis of 3-hydroxy-3-methylglutaryl-coenzyme A reductase gene (CaHMGR) was completed in *C. asiatica* and the 3D homology model generated showed 84.9% quality and its expression was higher in nodal region tissues followed by leaves, stem and root. The blast results of CaHMGR protein with genus *Panax* produced a high similarity value. Reports suggest that tissues in the nodal region might have an important role in terpenoid biosynthesis. Proteomic and SNP analysis of

Farnesyl diphosphate synthase from *C. asiatica* was also reported (Jawahar, 2017; Kalita *et al.*, 2015).

Apart from this gene, many other genes are involved in the terpenoid biosynthesis pathway for *C. asiatica* were cloned. Phytosterols play a role in membrane constituents, and as precursors for hormone biosynthesis in higher plants. Cycloartenol synthase is an intermediate in phytosterol biosynthesis. Kim and his team isolated the cDNA of oxidosqualene cyclase, which encodes cycloartenol synthase and also full-length cDNA of CabAS (*C. asiatica* putative  $\beta$ -amyrin synthase) was cloned and sequenced. Its expression in leaves was confirmed by northern blot analysis. But it was suggested to rename as dammarenediol (CaDDS) after inducing the CabAS gene. In the terpenoid pathway, two molecules of farnesyl diphosphate (FPS) are converted into squalene by squalene synthase (SQS), which is a common precursor of triterpenoid and sterol biosynthesis. Full-length cDNA clone of FPS and cDNA Squalene Synthase (CaSQS) was isolated and their sequence contains an open reading frame of 1029 nucleotides encoding 343 amino acids with a molecular mass of 39.6 kDa and 1248 nucleotides, which code for 416 amino acids with a molecular mass of 47.3 kDa respectively. A full-length cDNA clone encoding the glucosyltransferase for which the corresponding gene product was characterized using proteomic methods and cDNA sequence data. The transcriptome data of methyl jasmonate elicited *C. asiatica* leaves was used to identify the glycosyl transferase involved in asiaticoside formation. Four UGT candidate genes were selected and characterized by heterologous expression and found UGT3AH1 protein catalyzes the glycosylation of ursane-type triterpene and produce asiatic acid monohexoside (Costa *et al.*, 2017; Kim *et al.*, 2005a, 2005b; Kim *et al.*, 2017, 2009; Kim *et al.*, 2005b, 2005a; Mangas *et al.*, 2009).

## SEQUENCING BASED MARKERS USED IN *C. ASIATICA*

Sequence-based markers and next-generation sequencing (NGS) are useful to study the genetic system and simultaneous sequencing for hundreds of thousands of molecules produce large volumes of sequence data. Plant genome sequencing development started when the first genome (*Arabidopsis thaliana*) was completed in 2000. The whole-genome short gun assembly of *C. asiatica* has a size of about 430.217 Mb and it contains nine chromosome sequence information which was performed by National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand (Pootakham *et al.*, 2021). Medicinal Materials DNA Barcode Database (MMDBD; <https://rdccm.cuhk.edu.hk/mherbsdb/>) and NCBI GenBank have the DNA sequence information of *C. asiatica* submitted which include regions of ITS, matK, rbcL, rps16, trnD-trnT, trnH-psbA, trnG, trnK-rps16, trnL, ycf5 and genome short gun assembly. ITS 2 DNA Barcode was sequenced and identified as a valuable marker for differentiating the *C. asiatica* from its adulterants (Xiao-chen Chen *et al.*, 2012; Sarwat & Yamdagni, 2016). The NCBI Sequence Read Archive (SRA) (<https://www.ncbi.nlm.nih.gov/sra>) contains 19 biosamples of *C. asiatica* as

of 9<sup>th</sup> March 2021 that include RNA sequence of leaf, stem, root tissues of *C. asiatica* (9 submissions), miRNA sequence of *C. asiatica* leaf (4 submissions), 454 sequencing of *C. asiatica* elicited by methyl jasmonate (1 submission), transcriptome sequence of *C. asiatica* (1), transcriptome analysis of root (3 submissions) and leaf (1 submission).

Sangwan *et al.*, conducted a broad analysis of *C. asiatica* leaf transcriptome sequence assembly and gene ontology prediction analyses. By Illumina (Solexa sequencing technology), paired sequencing of the three RNA pooled samples leaf transcriptome of *C. asiatica* produced 7.07 GB of sequence data in fastq format with over 41,922 important identities of transcripts. This study produced good coverage of the transcriptome and was useful to find out major pathway genes. 71 different transcription factor types have been found in the transcripts. Zinc finger family transcription factor class was found in large numbers (424) followed by F-Box Homeobox, myb WD40 repeat family, BHLH, AP2, GRAS, GATA etc. Seventeen gene sequences involved in the secondary metabolic pathways were identified. The real-time PCR (qRT-PCR) analysis was conducted in stem, leaf, seed and root tissues of the plant to confirm the bioinformatics practices. From this, leaf tissue was shown a higher level of expression of all the secondary metabolite genes than other plant tissues investigated. This study represents the major report on *C. asiatica* transcriptome based gene resource development (Sangwan *et al.*, 2013).

A small molecule of endogenous RNA (21–24 nucleotide) called miRNA could be used as a marker for the identification of gene of interest as well as helpful in the breeding approach. miRNA plays a role in biological processes and post-transcriptional regulation (Xuemei Chen, 2005; Gangadhar *et al.*, 2014). miRNA based marker is evolving as a new marker of choice because of its highly conserved nature. miRNA based markers already developed for genotyping various plants including medicinal plants viz *Moringa oleifera* (Pirro *et al.*, 2016), *Silyum marianum* (Ražná *et al.*, 2015) etc. PMRD (plant microRNA database) (Z. Zhang *et al.*, 2010), miRNEST (Szcześniak *et al.*, 2012), mPC (MicroPC) (Mhuanong & Wichadakul, 2009) and miRBase (Griffiths-Jones *et al.*, 2007) are some of the miRNA databases which include published miRNA sequences and annotations (B. Zhang & Wang, 2015). *C. asiatica* miRNA sequences were submitted to the NCBI SRA database from JNTBGRI. miRNA primers (miR477e, miR156d, miR169f, miR397 and miR172) which are indicative of plant stress were used for Real-Time RT-PCR in *in vitro* grown *C. asiatica* samples. An initial downregulation was observed in miR169f and miR172 and overexpression in miR477e, miR156d and miR397 from 30 to 45 days cultures. This upregulation of miR156, miR172 and miR397 in *C. asiatica* could be due to the correlation with the enhancement in biomass, levels of auxins/cytokinins and tolerance to cold stress respectively (Jisha *et al.*, 2019). Another source for molecular marker development, the Chloroplast genome of *C. asiatica* was sequenced by Chan Li *et al.* The sequenced Chloroplast genome contains 62.30% of AT content and 113 genes (4 rRNA genes, 79 protein-coding genes, 30 tRNA genes) (C. Li *et al.*, 2020).

## CHROMOSOME AND POLYPLOIDY STUDY

*C. asiatica* karyotype was studied in ten ecotypes (Das & Mallick, 1991). The chromosomal variation was found in secondary constriction, total chromosome length and volume, total F% value which might be due to duplication and translocation that occurred in the chromosome during an early stage of evolution. Apart from the normal chromosome  $2n = 18$ , some ecotypes shown additional B chromosome and also a reduction in the size of autosomes. Tetraploid was first reported in *C. asiatica* ( $2n = 36$ ) by Kokubugatai *et al* (Kokubugata *et al.*, 1998). Polyploid breeding was tested in *C. asiatica*, in which a saturated paradichlorobenzene (pDB) antimetabolic agent effectively induced the autotetraploid. The induced plant produced a higher amount of asiaticoside and increased plant size. Genetically stable mixoploid with higher phytomass and triterpenoid content was produced by colchicine 0.025–0.1% concentration for 12–24 hr induction in *C. asiatica* (Dalave & Apparao, 2007; Kaensaksiri *et al.*, 2010). The study conducted by Rakotondralamo *et al.* (2013) confirmed the presence of tetraploidy of *C. asiatica* population with 36 chromosomes and diploid population which shown evidence for polyploidy complex nature.

## CONSERVATION AND CULTIVATION

Biodiversity nowadays is changing in an uncontrolled way, the most cause of these changes are due to changes in climate, pollution, unsustainable harvesting of natural resources, increase in the human population followed by an increase in consumption rate. Due to these kinds of disturbances, great numbers of plant resources are lost forever. Conservation involves the preservation of available genetic diversity. Many researchers believe that medicinal plants collected from wild habitats than cultivated have more active secondary metabolite content. But continuous use of wild resources will lose the genetic diversity in that habitat so conservation plays a role to preserve this. Around the world, many conservation programmes are initiated widely.

In India, for the collection and conservation of medicinal plants, many institutes are involved under Government schemes. Those institutes include institutes working under AYUSH, state forest departments under the Ministry of Environment and Forests (minor forest products), CSIR under the Department of Science and Technology, Defence Research and Development Organization and apart from these agriculture universities, the Department of Biotechnology, CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP), Directorate of Medicinal and Aromatic Plants Research (DMAPR), Central Council for Research in Ayurveda and Siddha under Ministry of Health and Family Welfare, National Bureau of Plant Genetic Resources (NBPGR), New Delhi, Ministry of Agriculture, Farmers Welfare under the support of Indian Council of Agriculture Research (ICAR), G-15 project funded by Department of Biotechnology, three medium-term conservation facilities were created in India, viz. CIMAP in Lucknow, JNTBGRI in Thiruvananthapuram and NBPGR in New Delhi

for the conservation (Bandara *et al.*, 2011; Bhau, 2012; Devkota & Jha, 2010; V. Gupta, 2018; A. Kumar & Jnanesh, 2017; Lal *et al.*, 2017; Mathur *et al.*, 2000; NMPB, 2014; Patel, 2015; Rao, 2016; Tandon *et al.*, 2009; Vinolina *et al.*, 2012). Several State/Central Government funded projects involving diverse medicinal plants conservation have been completed or are being carried out such as JNTBGRI has developed *ex-situ* conservation of the genetic resource of selected medicinal plants, constructed field Gene Banks and seed bank and practice assessment of intraspecific genetic variability using molecular markers; CSIR-Central Institute of Medicinal and Aromatic Plants Research Centre (CIMAP), Hyderabad, India, selected medicinal plants of the Deccan plateau region and maintained them in a field gene bank and seed bank. Many medicinal plants including *C. asiatica* are rich in the Western (3500 species) and Eastern (>1500 species) Ghats (JNTBGRI, 2016; NMPB, 2014).

For the cultivation of *C. asiatica*, the nodular part of the stem/runner clonal propagation was suggested. This method was acceptable and succeeded when they are grown under favorable conditions but this herb is still considered a wild plant and has not undergone domestication widely. In the case of seed germination is at a warm environment (25–30 °C), *C. asiatica* germination percentage in two to three months of old seed was recorded as 82% than the fresh seed. Released varieties of *C. asiatica* by CSIR-CIMAP called CIM Medha, RK-2, CA-7 and CA-4 were suggested for commercial cultivation. Majja Poshak, Subhodak, RK1, RK2 are also developed as cultivable varieties. Fresh herbage yield (12,331 kg/ha) and dry herbage yield (2,113 kg/ha) of high-yielding cultivar Vallabh Medha of mandukparni variety was identified by ICAR, India and also released Arka Prabhavi (CA-13) documented higher Asiaticoside content (>3%) (ICAR, 2021b), Arka Divya (CA-1) with broadleaf, mineral and vitamin A (32.33mg/100g) (ICAR, 2021a). Gotu kola cultivation through an aquaponic facility was exhibited at Crop Diversification Center South (CDCS), Canada which shows chlorosis in early stages, but it recovered subsequently. A study conducted on co-cultivation of *C. asiatica* with *Piriformospora indica* in pot experiments produced improvement in growth promotion and stress tolerance; this gives a good sign for a new way of cultivation. *C. asiatica* of 16 accessions collected from India screened for the ideal condition for growth. They identified CaBp and CaCl accessions produced high herbage and asiaticoside yields under full light and shading conditions; CaShT was recognized for its very high herbage and asiaticoside. National Medicinal Plants Board, India recommended that for the propagation and cultivation of *C. asiatica*, 300 kg rooted suckers are needed to plant one hectare of land and the maturity period is around 90 days to harvest. The harvest obtained in a year is 10–12 t/ha yield which is obtained by 3 harvests. For the cost of cultivation for one hectare Rs. 40000/- may be required (Jisha *et al.*, 2019; NMPB, 2014; Satheesan & Sabu, 2019).

## BIOLOGICAL DATABASES

Biological databases would be the most preferred choice to gather available genetic information. Many databases are

helpful to provide data on medicinal plants. Notable databases include the National Center for Biotechnology Information (NCBI) GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>), International Nucleotide Sequence Database Collaboration (INSDC; <http://www.insdc.org/>), PIR (Protein Information Resource; <https://proteininformationresource.org/>), PDB (Protein Data Bank) (<http://www.rcsb.org/pdb/home/home.do>), DNA Data Bank of Japan (DDBJ; <http://www.ddbj.nig.ac.jp/>), KEGG (Kyoto Encyclopedia of Genes and Genomes) (<https://www.genome.jp/kegg/>), MMDB (Molecular Modeling Database) (<http://www.ncbi.nlm.nih.gov/Structure/MMDB/mmdb.shtml>), European Molecular Biology Laboratory's European Bioinformatics Institute (EMBL-EBI; <http://www.ebi.ac.uk/embl/>), UniProtKB/Swiss-Prot (<http://www.uniprot.org/uniprot>) and Entrez Protein Database (<https://www.ncbi.nlm.nih.gov/protein>) (Amid *et al.*, 2012; Barker *et al.*, 1998; Benson *et al.*, 2000; Berman *et al.*, 2002; Boutet *et al.*, 2016; J. Chen *et al.*, 2003; Kaminuma *et al.*, 2010; Kanehisa & Goto, 2000; S. Kumar & Shanker, 2018; Wheeler *et al.*, 2007).

Some of the web resources available are helpful to gather the knowledge of medicinal plants such as Indian Medicinal Plants Database (<http://www.medicinalplants.in/aboutfrlhtdb>), The HerbMed (<http://www.herbmed.org>), The Indian Medicinal Plants, Phytochemistry, and Therapeutics (IMPPAT; <https://cb.imsc.res.in/imppat/home>), Medicinal Materials DNA Barcode Database (MMDBD; <http://www.cuhk.edu.hk/icm/mmdbd.htm>), Natural Products Alert (NAPRALERT; <https://www.napralert.org/about>), Indonesian medicinal plants database (<http://herbaldb.farmasi.ui.ac.id>), The Natural Medicines Comprehensive Database (<http://www.naturaldatabase.com>), Natural Products CARE (NPCARE; <http://silver.sejong.ac.kr/npcare>) and Super Natural II ([http://bioinfapplied.charite.de/supernatural\\_new/index.php](http://bioinfapplied.charite.de/supernatural_new/index.php)) (Banerjee *et al.*, 2015; Choi *et al.*, 2017; Hsu, 2002; S. Kumar & Shanker, 2018; Lou *et al.*, 2010; Loub *et al.*, 1985; Mohanraj *et al.*, 2018; Wootton, 2002; Yanuar *et al.*, 2011).

## CONCLUSION REMARKS

*C. asiatica* has been recognized as an imperative drug in various Pharmacopoeia of the world viz. India, Germany, Europe and the People's Republic of China. Even though it was a highly demanded and traded plant according to Indian Medicinal Plants Database, the source for trading mainly from the wild, so ultimately it would lead to overexploitation and hence cultivation and breeding strategies are to be developed to improve the availability. Molecular marker-assisted breeding is popular in crop plants but in medicinal plants only a few reports are available. DNA markers would be helpful in marker-assisted breeding and validating the medicinal plant specimens required for making pharmaceutical products (Azizi, 2010; Choudhri *et al.*, 2018; Dong *et al.*, 2017; Prakash *et al.*, 2017; Sharma *et al.*, 2018; Zeng *et al.*, 2010). WHO suggests correct identification and quality assurance required for traditional medicine. Molecular markers can be used for such testing and biological standardization. Utilization of genetic markers for studying physiology, genetics, taxonomy etc are common among food crops and horticultural plants but lately, they are



being used in pharmacognostic characterization to locate the gene, to improve as well as to authenticate the plant varieties, genome analysis, genetic characterization, fingerprinting, understating the heritability and phylogenetic analysis to predict evolutionary theories in medicinal plants. Gene microarray study in *C. asiatica* helped to understand the bioactivity and molecular mechanism (Belwal *et al.*, 2019; Chavan *et al.*, 2006; Coldren *et al.*, 2003; Srivastava *et al.*, 2009; Tan, 2017). Various genetic analyses implementation would be helpful in better-quality accessions selection and new cultivar development in *C. asiatica*. This review shows the requisite research path on its genetic research gap and also provides awareness on the requirement for its improvement in conservation, cultivation and genetic improvement of this medicinal plant.

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## CONFLICT OF INTEREST

It is authors declared that no conflict of interest in the publication of this article.

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