Genetic Diversity in *Commiphora wightii* (Arn.) Bhandari (Guggul): An Assessment of Populations in Conservation Sites of Kachchh Region (Gujarat) of India

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ABSTRACT

Introduction: Commiphora wightii (Arn.) Bhandari (Guggul) is native to semi-arid to arid zones. Its oleoresin gum is used for both medicinal and non-medicinal applications. Reportedly, the species faces highdegree conservation threats. A network of Four Medicinal Plants Conservation Areas (MPCA) was carved out in Kachchh region- under financial support from National Medicinal Plants Board. The species also occurs in sanctuary and protected by default. It is considered important to assess genetic diversity of these conserved populations. Methods: A total of 38 accessions of Guggul leaves were collected from five study sites and the whole genomic DNA was extracted for each sample. Genetic study was carried out using ISSR technique. Scorable bands were obtained for 14 out of 40 primers tested. A total of 49 bands were obtained of which 20 were polymorphic in nature. UPGMA dendrograms were constructed for individual sites and also a collective dendrogram for all the study sites. Results: All the 8 accessions of Tharawada MPCA were observed to share a high similarity coefficient. Among rest of three sites, genetic diversity was seen to be narrow. The accessions obtained from NSWS displayed good diversity. These accessions were also noted to be distant to the populations captured in the MPCA sites. Conclusions: Established MPCA sites capture the available gene pool of Commiphora wightii of Kachchh region. Since NSWS populations are distant to rest of the region, it is suggested to create another site for this purpose. It is desirable to assess the genetic diversity in rest of the MPCA sites.

Key words: Guggulu, Gene Pool, In-situ conservation, Medicinal Plants Conservation Area, MPCA, UPGMA.

INTRODUCTION

Commiphora wightii (Arn.) Bhandari (Syn. Commiphora mukul Hook Ex. Stock, Balsamodendron mukul Arn.) of Family Burseraceae is a perennial shrub found in the rocky tracts of semi-arid to arid forests of Indian sub-continent. The shrub is characterized by small thorns, sturdy, and short trunk with papery bark. Its crooked, knotty branches terminate into sharp spines and trifoliate glandular leaves.1 The plant is balsamiferous in nature and contains a resinous substance in the resin ducts that run along the trunk and branches. A yellowish oleoresin gum termed as Guggulu in Sanskrit is secreted in response to injury. The plant, hence, is often referred to as Guggul tree. The exudate contains resin exceeding 61%, polysaccharidic gum 29% and other constituents like sterols, diterpenes and higher alcohols.2

The gum resins of *Commiphora wightii* is recommended for treatment of arthritic conditions, lumbo-sacral pain, and lipid disorders etc.³ The oleoresin gum of the species attained greater medicinal and commercial importance with unravelling of its anti-hyperlipidaemic properties and launch of a formulation standardized to E and Z sterones.⁴ In addition, the exudate of *Guggul* is also used for perfumery, calico-printing, fabric dying. Due to presence of oleoresins, even the green twigs burn easily. Hence, the species also

finds place in fuel woods. All these uses bring about tremendous pressures of harvesting.⁵

Sustainability threats

Guggul is posed by many of sustainability threats. Poor rates of seed setting,6 slow growth rate due to the hostile conditions prevailing in rocky terrains of arid and semi-arid regions⁷ and increasing presence of invasive species⁵ are three primary factors of concern. Over and above, the collection of oleoresin gum is unsustainable since the injury leads to infection by Xanthomonas axonopodis- which causes gummosis in the plant.8 Traditional harvesting practices induce the infection by use of knives dipped in old *Guggulu* solutions. The spread of infection is further augmented by the multiple incisions on the trunk and main branches. In consideration to cumulative impacts of these factors and prevailing rate of decline in the populations in the native habitats, Guggul was placed in vulnerable category during 1998, and under Critically Endangered (CR) category by IUCN in the year 2014.9

In response to these challenges, the Government of Gujarat carved out 4 Medicinal Plants Conservation Areas (MPCAs) in Kachchh forest circle for conservation of the species under financial support from the National Medicinal Plants Board. ¹⁰ In addition, protected areas in the region also contain populations of the species which are conserved by default and Narayan Sarovar Wildlife Sanctuary (NSWS) in Kachchh West Forest division is one of them. ¹¹



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Conservation of genetic diversity

Genetic diversity or in other words the gene pool possessed by a population represents the heritable variation within and between populations of any given species of living organisms. Conservation of genetic diversity in the plant species is considered important for welfare of human beings.¹² Genetic diversity accounts for the probability of a population to adapt to changes in environment and evolve and therefore understanding of intra specific genetic diversity has become a core issue of conservation genetics.¹³ In view of the emphasis on chemical features, intra-species genetic diversity attains greater importance for medicinal and aromatic plants.¹⁴ Thus, it may be construed that the process of conservation needs to include preservation of intra-species genetic diversity in general.

The assessment of genetic diversity in the plant populations is performed using various techniques such as morphological, Cytological, biochemical (allozyme) characterization, and Molecular (or DNA) marker analysis. The most widely used technique for studying the genetic diversity is molecular markers analysis, owing to its hyper variability, better genomic coverage, high reproducibility and being neutral and free from environmental fluctuations. 15 Among the various molecular markers, microsatellites are remarked as being among the most important tool in studying the genetic diversity. Microsatellites are very short (usually 10-20 base-pair) stretches of DNA that are "hypervariable", expressed as different variants within populations and among different species.¹⁶ Inter Simple sequence Repeats (ISSR) technique accesses variation in the numerous microsatellite regions dispersed throughout the genome and thus helps to bypass the challenge of characterizing individual loci that Simple Sequence Repeats (SSR) approach require. Heritability studies of ISSR locus demonstrates highly close approximation to classic Mendelian ratios.¹⁷ High polymorphism as compared to other molecular markers makes ISSR ideal for genetic diversity studies. 18,19

In case of Guggul, protocol for isolation of genomic DNA was standardized.²⁰ Genetic variability in the species reported in earlier reports for samples drawn from selected areas.^{21,22} However, the genetic diversity captured in the conservation sites was not assessed and reported. Therefore, a study was carried out with a focus on conserved populations in Kachchh region.

Objective of the study

To assess the genetic diversity of populations *Commiphora wightii* (Arn.) Bhandari captured in four MPCA sites and one of the Wildlife sanctuaries in Kachchh forest circle.

MATERIALS AND METHODS

Study/ Sample sites and details

Randomly collected samples of Guggul from 5 sites were used for this study. 6-8 leaves were collected from each of the shrubs selected at random in the study site. The collected leaves were soon transferred into sampling pouches containing Silica Gel Orange with Moisture Indicator (Sigma-Aldrich Cat. No: 13767). Sample collected from each tree is counted as one accession. The collected samples were then transported to the Dabur Research and Development Centre for further study.

A total of 38 accessions were collected from the targeted sites and assigned an analysis code for the study. The details of samples along with the accession codes is represented in Table 1.

DNA Extraction, PCR and electrophoresis

DNA was isolated from the collected accessions using standard procedure of Qiagen DNeasy plant DNA isolation mini kit (Qiagen, USA) and stored at -20°C. ISSR (Inter Simple Sequence Repeats)

fingerprinting PCR was performed using primers synthesized by Sigma Aldrich, Bangalore; in a reaction volume of 50μ l using Taq PCR master mix (Qiagen, USA). The annealing temperature was optimized to be 42°C for AT rich ISSR primers, 52 °C for GC rich ISSR primers. The PCR program had an initial cycle of 5 minutes at 94°C followed by 45 cycles of 1 minute at 94°C, 1 minute at respectively optimized annealing temperature and 1 minute at 72°C. The final extension was performed at 72°C for 10 minutes. Amplicons thus obtained were subjected to agarose gel electrophoresis. 1.5% (w/v) Agarose was dissolved in 1X TAE buffer. 0.5 μ g of Ethidium Bromide per ml of gel solution from stock solution (10mg/ml) was added. Gel was poured in the casting tray with comb and allowed to set. Sample for electrophoresis were prepared by adding 1μ l of 6x gel loading dye for every 5μ l of DNA solution and 5- 12μ l was loaded in each well. The run was completed at a constant voltage of 80V.

Visualization and data analysis

Gels were visualized with AlphamagerEC imaging system (Protein Simple, CA, USA) and analysis of bands was performed by Alphaview software version 3.4 (Protein Simple, CA, USA). The presence of an amplified product (band) in each position was recorded as 1 and absence as 0. The fragment size of each band in bp (base pairs) was calculated using GeneRuler 100bp DNA Ladder (Thermo Scientific). Banding patterns produced by different accessions were then compared to determine similarity or dissimilarity of fingerprints.

Similarity matrix (Dice, 1945) generated using the band data was converted to similarity coefficient distances.²³ Using the distance matrix, Cluster analysis was done by constructing dendrogram by Unweighted Pair Group Method with Arithmetic Mean (UPGMA). The computer package NTSY Spc 2.2was used for cluster analysis.²⁴ Similarity coefficients were tested by applying SIMQUAL, SAHN procedures in NTSY Spc 2.2.

RESULTS

Scorable bands were obtained from 14 out of 40 primers tested. A total of 49 bands were obtained, out of which 20 were polymorphic (40.88%). Average bands obtained per primer were 3.5. The primers used along with their sequence, total band obtained, polymorphic bands and their percentage are listed in the Table 2.

Samples drawn from Narayan Sarovar Wildlife Sanctuary

A total of 8 samples were collected from the site. The dendrogram of the site is shown in Figure 2. Six accessions (accession no's CMB-1 to CMB-8) in the site display good diversity within the samples.

It may be observed that the accessions are distinctly divided into 2 clusters separated by a similarity coefficient of 0.92. Accessions within these clusters shared a similarity of around 0.98. The accessions, CMB-1, and CMB-4 as well as the accessions CMB-5 and CMB-7 are closely related by the highest similarity coefficient of 1.

Samples drawn from Guggliyana MPCA

The UPGMA dendrogram of the samples drawn from Guggliyana MPCA site (Dayapar South Range, Kachchh West Forest division) is shown in Figure 3. Accessions CMG-1 to CMG-8 represent the populations of Guggliyana MPCA. They showed an average similarity coefficient of 0.98 within the accessions, although one accession CMG-6 is most distant. It shared a similarity coefficient of 0.96 with others. CMG-1, CMG-3 and CMG-4 shared highest similarity of 1.

Accessions from Mangwana MPCA

Figure 4 shows the dendrogram for the six samples drawn from Mangwana MPCA (Nakhatrana Range, Kachchh West Forest

division). These accessions too share a high-similarity coefficient of 0.98. However, one accession, CMM-4 is separated by a coefficient of 0.97. Remaining accessions formed two clusters with highly similar groups sharing similarity coefficient of 1.

Accessions from Tharawada MPCA

Eight accessions were collected from Tharawada MPCA (Bhuj North Range, Kachchh East Forest division) and were coded as CMT-1 to CMT-8. However, all these samples were observed to share high similarity coefficient of 1 and formed a single cluster. It may be concluded that, these accessions do not represent any genetic variance. (Figure 5)

Accessions from Ler MPCA

High similarity was also observed within 8 accessions (CML-1 to 8) from Ler MPCA (Bhuj South Range, Kachchh East Forest Division) with 3 accessions diverging at a similarity coefficient of 0.98 (Figure 6).

Collective dendrogram of all accessions and comparison

The UPGMA dendrogram for all the accessions is presented in the Figure 7. It may be observed that, the samples are divided into two distinct clusters.

Cluster-A: Genetic diversity with similarity coefficient of 0.93 to 1.00 was found within the accessions in this cluster. However, 8 accessions of CMT were found to be monomorphic (with similarity coefficient of 1.00). Also, a narrow diversity (0.97-1.00) is observed between 8 accessions of CML with 5 monomorphic accessions.

A sub-cluster within Cluster-A is observed which covers all accessions of CMM with CMM-4 being closely similar to CMM-6. Five accessions of CMG formed a diverse group within this sub-cluster.

Cluster-B: This cluster is formed by a fairly diverse group of accessions with similarity coefficient of 0.92 to 1.0. It contains all the accessions of CMB.

DISCUSSION

This study on the genetic diversity of *Commiphora wightii* (Arn.) Bhandari has specific reference to conservation of the subject species.

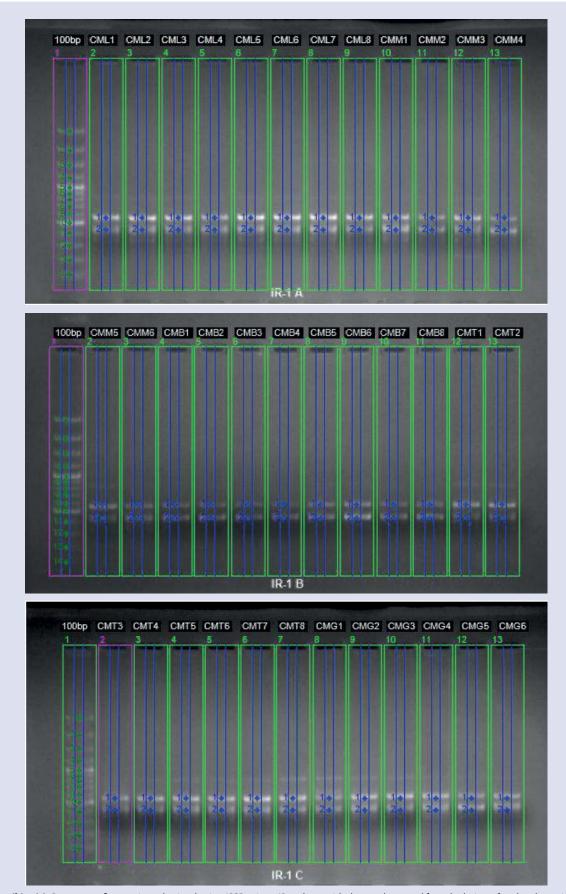
Agricultural resources offer for gene pool conservation under ex-situ conditions. Seed banks established for this purpose may represent different types of germplasm, cultivars, and landraces. On the contrary, genetic conservation of wild plant species mostly demand in-situ models in view of the ecologically specific requirements for each of the

Table 1: Sample details and accession codes.

Sites	Geographical details	Accession code and samples	
Ratipar Beat Dayapar South Range Narayan Sarovar WLS, Kachchh West Forest Division	23 °32' 22.77" (N) 68 °36'58.67" (E) Altitude: 75 m a.m.s.l	Analysis Code: CMB Samples: 8 Samples code: CMB-1 to 8	
Guggliyana MPCA Guggilyana Beat Dayapar South Range Kachchh West Forest Division	23 ° 33' 43.29" (N) 68 ° 57' 33.65" (E) Altitude: 130 m a.m.s.l.	Analysis Code: CMG Samples: 8 Samples code: CMG-1 to 8	
Mangwana MPCA Mangwana Beat Nakhatrana Range Kachchh West Forest Division	23 ° 11' 50.04" (N) 69 ° 21' 38.80" (E) Altitude: 156 m a.m.s.l.	Analysis Code: CMM Samples: 6 Samples code: CMM-1 to 6	
Tharawada MPCA Tharawada Beat Bhuj North Range Kachchh East Forest Division	23 ° 10' 49.80" (N) 69 ° 51' 23.27" (E) Altitude: 188 m a.m.s.l.	Analysis Code: CMT Samples: 8 Samples code: CMT-1 to 8	
Ler MPCA Ler Beat Bhuj South Range Kachchh East Forest Division	23 ° 10'49.80" (N) 69 ° 45'24.71 (E) Altitude: 169 m a.m.s.l.	Analysis Code: CML Samples: 8 Samples code: CML-1 to 8	

Table 2: Primers and obtained percentage of polymorphism.

Primer	Sequence	Total Bands	Polymorphic Bands	P (%)
ir-1	5'-AGAGAGAGAGAGAGC-3'	2	0	00.00
ir-2	5'-CACACACACACACAG-3'	3	1	33.33
ir-5	5'-CACACACACACACAA-3'	5	1	20.00
ir-7	5'-ACACACACACACACC-3'	3	2	66.66
ir-8	5'-CTCTCTCTCTCTCTG-3'	4	2	50.00
ir-10	5'-TGTGTGTGTGTGTGA-3'	1	0	00.00
ir-18	5'-GTGTGTGTGTGG-3'	2	1	50.00
ir-19	5'-GAGAGAGAGAGG-3'	4	0	00.00
ir-21	5'-GAGGAGGAGGC-3'	4	1	25.00
ir-22	5'-GTGGTGGTGGC-3'	4	0	00.00
ir-23	5'-CAGCAGCAGCAG-3'	2	0	00.00
ir-31	5'-GACGACGACGAC-3'	3	2	66.66
ir-33	5'-CACCACCACGC-3'	7	6	85.71
ir-34	5'-GAGAGAGAGACC-3'	5	4	80.00
Total		49	20	40.88
Average		3.5	1.4	40.00



Figures 1(a), 1(b), 1(c): Represents fingerprints obtained using ISSR primer IR-1 along with the overlays used for calculation of molecular weight GeneRuler 100bp DNA Ladder (Thermo Scientific).

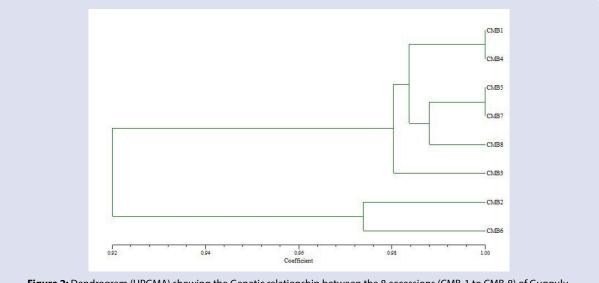


Figure 2: Dendrogram (UPGMA) showing the Genetic relationship between the 8 accessions (CMB-1 to CMB-8) of Guggulu.

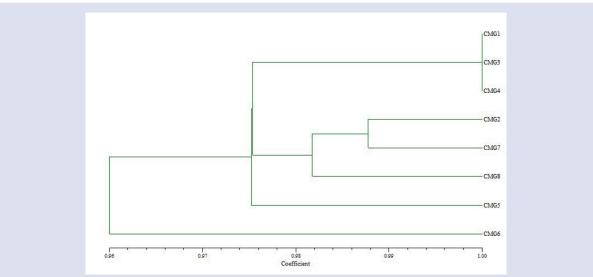


Figure 3: Dendrogram (UPGMA) showing the Genetic relationship between the 8 accessions (CMG-1 to CMG-8) of Guggulu.

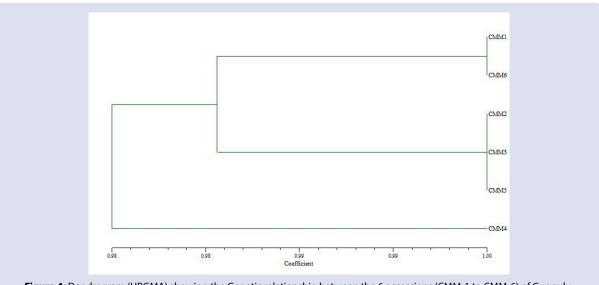


Figure 4: Dendrogram (UPGMA) showing the Genetic relationship between the 6 accessions (CMM-1 to CMM-6) of Guggulu.

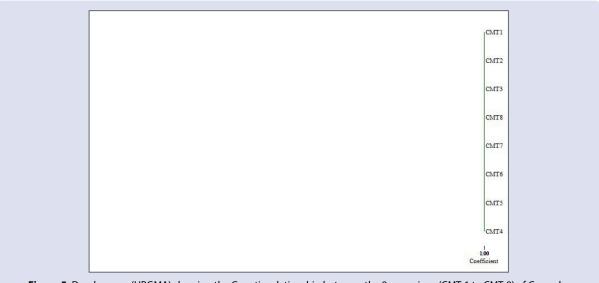
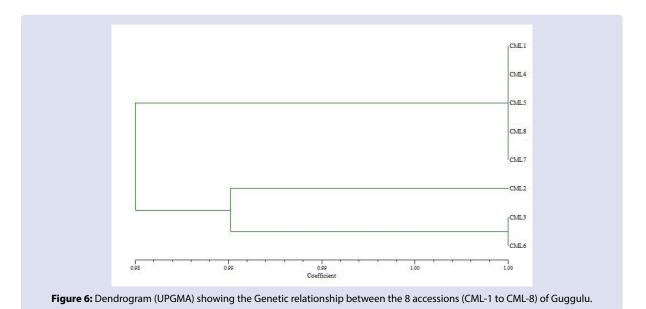


Figure 5: Dendrogram (UPGMA) showing the Genetic relationship between the 8 accessions (CMT-1 to CMT-8) of Guggulu.



genotype. Maintenance of ecosystems is a widely accepted paradigm for in-situ conservation for this purpose. However, this approach might not capture all the desirable traits for a focused conservation.¹⁴

Without consideration to genetic and chemical diversity, conservation of medicinal plants may remain truncated. In this context, the concept of Medicinal Plants Conservation Areas (MPCAs) provides an improved scope. Before the inception of this model, there were no formal or focused approaches for conservation of the category at species and genetic levels. Conceptualized during the year 1993, the model seeks to confer protection to threatened medicinal plants in a focused manner.²⁵ Under existing modalities, MPCAs may be carved out in both reserve and protected forest areas.

In comparison to conventional methods, MPCA model offers some specific advantages *viz*. (a) it is intentional in its approach and focused on a category of biological resources, (b) these sites can be established and managed in smaller areas of natural habitats. Hence, it would be possible to establish multiple sites for target species in different geoclimatic zones to capture the intraspecific genetic and phytochemical diversity, and (c) it provides for extensive ecogeographical studies including assessment of phytochemical and genetic profiling as part of groundwork - in an unwritten manner. Thus, the baseline research on the proposed site is critical to fulfil all the objectives of the MPCA model.

The sites selected for this study, are primarily MPCAs which were carved in reserve forest areas for conservation of Guggul. These sites provided for intentional preservation of natural populations in smaller areas of 50 ha each and are functional since the Year 2008. Since, these four units were carved out in different localities, it would be logical to assess the genetic polymorphism which was not done as a part of baseline research. For making the assessment comprehensive, the Guggul plants inhabited in NSWS which were conserved by default were also included in the study.

Ecological factors are considered to play an important role in determining the genetic structure in plant populations.²⁶ This phenomenon implies that; the polymorphic genetic structure may be encountered if; the samples are drawn from wider ecological conditions. In this study it was observed that; one of the MPCA sites (Tharawada) hosted single genotype without visible variance. In rest of the three sites, the genetic diversity was noted to be narrow. Though, the sample locations are different, they fall in common ecogeographical conditions. Hence, this kind of outcomes looks to be justified.

In contrast to the MPCA sites, the population in the wildlife protection site, NSWS, inhabits a unique genotype of Guggulu.

Overall, it may be stated that the genotypes available in the Kachchh region are well represented in the MPCAs and protection sites. The populations of Guggul in the Narayan Sarovar Wildlife Sanctuary offer a scope for designation of new MPCA in Kachchh region. Though the site falls in a protected area, carving out MPCA brings scope of focused interventions for habitat management including checks on invasive species. Further, it is reported that MPCAs for *Commiphora wightii* were established in other areas in the state. ^{10,25} Assessments of genetic diversity in these sites is highly desirable. Similarly, mapping the Guggul populations in all the MPCAs established for conservation of Guggul in the state, and also in the neighbouring states for phytochemical characteristics would strengthen the Guggul conservation process in a holistic manner.

CONCLUSIONS

A total of 38 samples of *Commiphora wightii* (Arn.) Bhandari- known as *Guggulu* in Sanskrit were taken up for assessment of genetic

polymorphism. Thirty of these samples represented the populations captured in four of the Medicinal Plants Conservation Areas (MPCAs) aimed at long-term, non-interventional conservation of the species. The genetic diversity prevailing in these four MPCAs was observed to be narrow. Rest of eight samples represented the populations contained in a wildlife sanctuary of Kachchh region. These samples were observed to be genetically distant to rest. The sampling site in Narayan Sarovar Wildlife Sanctuary offers potential for carving out the fifth MPCA so as to protect all the genotypes of *Commiphora wightii* in the region.

DECLARATIONS

Conflicts of interest

Authors declare no conflicts of interest.

Financial disclosures

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Research ethics

Necessary permissions were obtained from the concerned authorities for sampling in the study sites and the same is acknowledged.

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Author's contributions

All authors contributed equally to the design, data collection, analysis and drafting of the manuscript.

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ABBREVIATIONS

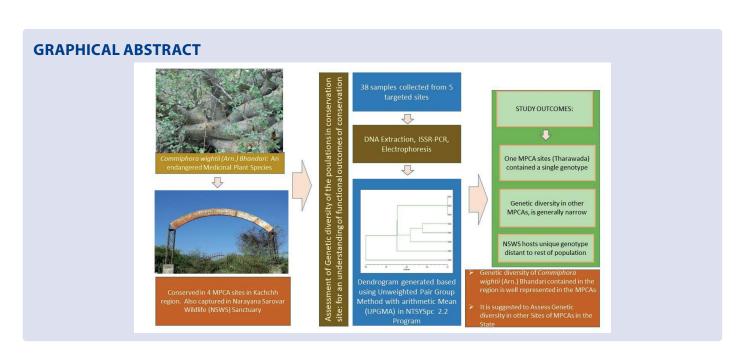
MPCA: Medicinal Plants Conservation Area; NSWS: Narayan Sarovar Wildlife Sanctuary, UPGMA: Unweighted Pair Group Method with Arithmetic Mean.

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ABOUT AUTHORS



Mr. Brindavanam is a graduate in Ayurvedic medicine (B. A. M. S.) and holds a postgraduation in medicinal plants. He joined pharmaceutical research in 1985 after a stint in clinical practice. His work spans across all the important spheres of research and development of herbal medicines. As Head of Bio Resources Development Group at Dabur Research & Development Centre, he played pivotal role in introducing four forest species to farming. He also worked on ancient Ayurvedic concepts of substitutions development- in favor of sustainable resource management of medicinal plants. Mr. Brindavanam is known for his skills in leading interdisciplinary research on medicinal plants. He contributed chapters in four reference books and monographs and published serval research communications. At present, he is pursuing his Ph. D program from Amity University after superannuating from corporate R & D services.



Dr Gurinderjit Goraya holds a doctorate in forestry and served in the Indian Forest Service for over 35 years. In this span of his experience, Dr Goraya worked in teaching, research and institutional development in addition to the forest management. He contributed to conservation of medicinal plants during his stint with Foundation for Revitalization of Local health traditions and promoted the National drugs depository for plant based drugs. Dr Goraya published several research papers, reports, other educational materials on medicinal plants. He co-authored two major analytical reports on trade of medicinal plants. He is vivid explorer of forest flora and is fond of trekking which he continues to pursue now also. In recognition to his contributions to the medicinal plants, floral diversity, Two plant species new to Science are named after him.



Prof. Singh holds doctorate in Insect Physiology and also master's degrees in forestry, remote sensing, sociology and a post graduate diploma in management. He served in Indian Forest Service and superannuated as Head of Forest Force, Government of Assam, India. As part of his career, he served as Dy. Director General of ICFRE, the nodal agency for forestry research in India. Prof. Singh worked extensively in Natural regeneration of Rain Forest species, carbon sequestration in Sal forests using remote sensing technologies. Prof. Sigh was also associated with project formulation and management of eco-development in the protected areas, assessment of biodiversity and forest productivity in Garo Conservation areas. Further, he was closely associated with conservation of threatened faunal species like Pygmy hog, Hispid hare, Bengal florican etc. He has published several research communications and presented in international conferences.



Amandeep holds a Master of Science in plant biotechnology and Master of Philosophy in industrial biotechnology. With over 14 years of professional experience, he is presently working as a molecular biologist in Dbur Research and Development Centre. His work largely involves with on detection of DNA variation in plants using molecular markers. Genetic diversity analysis for conservation of endangered medicinal plant species, root rhizospheric microbiome analysis of endemic high altitude plant species for identifying plant specific associated microbes, DNA barcoding-sequencing and species identification are other areas of core interest and focus. He has 11 research publications to his credit.



Ms. Ankita holds M. Tech degree in Biotechnology from Amity University and is currently associated with the Bio Resource Development Group, Dabur Research and Development Centre. Her work involves DNA Fingerprinting for medicinal plants resources used in Ayurvedic formulations and mapping intraspecific genetic diversity. Prior to this assignment, she worked with Q-Line biotech Pvt Ltd, as QC In-charge and Arbro Pharmaceuticals as Junior Analyst, accounting for a total experience of about 6 years.

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