

Genomic Resource Generation in Medicinal and Aromatic Plants

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Plant genomic resources are genetic material of actual or potential value which can be utilized for the improvement of specific traits in Agri-horticultural crops. The generation of genomic resource in medicinal plants is important because they contain bioactive compounds or secondary metabolites important for human health. The demand for these compounds is increasing due to their application in herbal medicine. The recent “omics” techniques have made generation of genomic resources much efficient and cost effective. The improvement in sequencing technology from 2nd generation (NGS) to 3rd generation has reduced sequencing cost and thus brought many more crop genomes within range of analysis. The Next Generation Sequencing (NGS) based whole genome and transcriptome sequencing in medicinal and aromatic plant has played a vital role in generating genomic resources for effective conservation, crop improvement and better understanding about secondary metabolite biosynthesis in medicinal and aromatic plants. In present review, the progress of generating genomic resources such SSR resources, EST-SSR resources, transcription factors, transcriptome analysis, and whole genome sequence analysis in selected medicinal and aromatic plants has been updated, which may be further utilize in medicinal and aromatic plant improvement programs.

Key Words: Aromatic plants, Genomic resource, Medicinal plants, Molecular markers Transcriptome, Whole genome sequencing

Introduction

Medicinal and aromatic plants are very important because they are rich sources of secondary metabolites or bioactive compounds required for production of herbal medicines. The affordability, availability, compatibility, and acceptability of medicinal plants have made them an important element in the primary health care. Over 70% of the population of developing countries relies upon medicinal plants for their treatment and primary care (Jeelani *et al.*, 2018). Medicinal plants have been used for centuries to treat and prevent different diseases. Different secondary metabolites or bioactive compounds derived from the medicinal plants used for producing medicines due to diverse medicinal properties such as anti-inflammatory, immunomodulatory, anticancer, cardiovascular, antimalarial, and antimicrobial.

The present review focuses on four important medicinal and aromatic plants, *Tinospora cordifolia* (Giloe), *Andrographis paniculata* (Kalmegh), *Vetiveria zizanioides* (Vetiver grass), and *Bunium persicum* (Kala jeera). The *Tinospora cordifolia* is a deciduous shrub, belongs to Menispermaceae family (Spandana *et al.*, 2013). In the Ayurveda, this plant is recorded as having bitter, pungent, and astringent tastes (Raghu

et al., 2006). *T. cordifolia* has been reported to have various important medicinal properties *viz.*, anti-oxidant, anti-hyperglycaemic, anti-stress agent, anti-carcinogenic, anti-spasmodic, anti-allergic, anti-leprotic, immunomodulator, anti-microbial (Jeyachandran *et al.*, 2003; Kalikar *et al.*, 2008; Khan *et al.*, 2020; Singh *et al.*, 2003; Asthana *et al.*, 2001; Desai *et al.*, 2002; Rajalakshmi *et al.*, 2009; Ahmad *et al.*, 2015). The *Andrographis paniculata*, belongs to Acanthaceae family, and commonly known as chireta (Chandrasekaran *et al.*, 2009). The plant contains a diterpenoid andrographolide which is bitter in taste, and responsible for the therapeutic interest of the plant. The several pharmacological activities of the plant has been reported such as cytotoxicity, antioxidant, antimicrobial, anti-inflammatory, immune-stimulant, antidiabetic, anti-infective, anti-angiogenic, hepato-renal protective, and insecticidal activities (Okhwarobo *et al.*, 2014). *Vetiveria zizanioides* L. Nash, which is a perennial grass, commonly referred as Khus, and belongs to the Poaceae family. The roots of the plant produce a fragrant and volatile oil content that is in high demand in the perfumery, and cosmetic industries (Sethi *et al.*, 1968). *Bunium persicum* (Boiss.) Fedtsch., commonly known as *Kala*

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jeera, is an important aromatic and medicinal plant from Apiaceae family, grows mainly in cold temperate regions of Central Asia and Northern India. Due to the high amount of aroma and essential oil present in the plant, *Kala jeera* is industrially important.

The advancements in genomic technologies have made generation of genomic resources in medicinal and aromatic plants easy and also to improve the desired traits or secondary metabolites production. Genomic resources such as genomic SSR (Simple sequences Repeats), ESTs (Expressed sequence tags), transcription factors and small RNA etc has been generated using technologies such as transcriptome and whole genome sequencing in some medicinal plants (Singh *et al.*, 2014; Singh *et al.*, 2016; Sun *et al.*, 2019; Kumar *et al.*, 2020; Bansal *et al.*, 2022).

1. Approaches Used to Generate Genomic Resources

Crop improvement goals are shifting toward a trait-oriented approach as agriculture becomes more specialised and location-specific. To achieve these goals, it is crucial to both conserve and make use of the genetic diversity that is already present. Generating genomic resources can significantly improve the use of PGRs (plant genetic resources). Due to omics techniques, the development of genomic resources is now possible in less time and in cost effective manner. Few of the genomic approaches which are being used for the generation of genomic resources in medicinal and aromatic plants (Fig. 1) are discussed below:

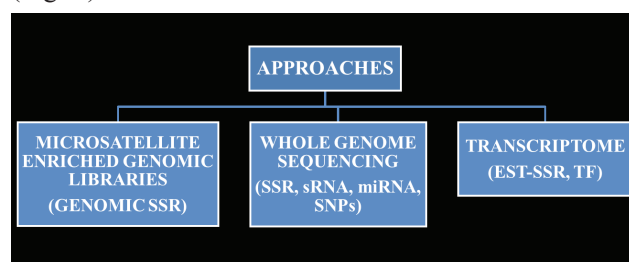


Fig.1. Approaches commonly used for generation of genomic resources in medicinal and aromatic plants.

1.1 Microsatellite Enriched Library

In this approach, the microsatellite containing the DNA region of the genome is hybridized using microsatellite repeat specific probes, the genomic DNA is fragmented/digested by either restriction digestion or sonication (Kandpal *et al.*, 1994; Edwards *et al.*, 1996; Fischer and

Bachmann, 1998). This is relatively simple, robust, low cost, and reproducible in comparison to other methods. The method has been used to generate genomic SSRs in medicinal plants such as *A. paniculata*, and *T. cordifolia* (Kumar *et al.*, 2020, Paliwal *et al.*, 2016).

1.2 Transcriptome Sequencing

RNA sequencing (RNA-seq) based on next-generation sequencing (NGS) platform, enable the simultaneous acquisition of sequences for both gene discovery and transcript identification relevant to biological processes. This approach is appropriate for those organisms for which genomic sequences information's are not available (Ward *et al.*, 2012). In recent years, de novo transcriptome has appeared as a powerful technique to identify genes involved in the biosynthesis of different secondary metabolites of medicinal plants (Huang *et al.*, 2012; Hyun *et al.*, 2012; Singh *et al.*, 2016).

1.3 Whole Genome Sequencing

The ability to sequence an organism's entire genome with new NGS technology at a lower cost and in less time has become one of the key discoveries in the field of "omics," even though "Sanger sequencing" has remained the standard for decoding genomes for several decades. Earlier, even sequencing a small genome would have required a multi-institutional collaborative effort and substantial funding. The advancement of NGS technologies has greatly increased the cost-effectiveness, speed, and efficiency of genome sequencing. The genome sequencing of some medicinal plants such as *A. paniculata*, *Ocimum tenuiflorum*, and *Artemisia annua* is available using NGS platform (Upadhyay *et al.*, 2015; Shen *et al.*, 2018; Sun *et al.*, 2019).

1.4 Genome-wide Association Studies (GWAS)

Genome-wide association studies (GWAS) have become a preferred method due to ongoing advancements in sequencing technologies and concerted community effort, especially when resequencing is carried out after the assembly of the reference genome or when a high-density genotyping array is made available (Michael and Jackson, 2013). This approach has allowed to find the genomic variations linked with either molecular or biochemical phenotype, and traditional agronomic phenotypes. These associations could be used to accelerate the crop improvement programs. The genome wide study has been done in *Matricaria recutita*, a medicinal plant (Otto *et al.*, 2017).

1.5 *Small RNA*

Small RNA, cis acting regulatory elements and intergenic regions which are part of intron region (non-genic region), also gaining the importance as genomic resources. Small RNAs play an important role in stress management in plants. The small RNAs has been discovered in medicinal plants such as *Panax ginseng*, *Dendrobium huoshanense* (Wu *et al.*, 2012; Wang *et al.*, 2022).

1.6 *Single Nucleotide Polymorphism (SNP)*

Identification of allele variations in PGRs, which can be obtained by highly reliable DNA-based markers such as SNPs. SNP provides better potentials for studying PGRs management in several ways, including cultivar identification, genetic diversity assessment, genetic map construction, and marker assisted breeding (Ganal *et al.*, 2009). This is because the SNP is more readily available and stable during inheritance than other markers, such as SSRs. The SNPs has been reported in medicinal plants such as *M. recutita*, and *Crepidiastrum denticulatum* (Otto *et al.*, 2017; Dang *et al.*, 2019).

2. Genomic Resources Generated in Medicinal and Aromatic Plants

2.1 *SSR Markers Generation through Enriched Genomic Library*

SSRs are also known as microsatellites, which are short tandem repeats of nucleotides (1-10) and distributed throughout the genome. Due to codominant in nature, multi allelic, high reproducibility and cross transferability, the SSR markers are one of the choicest marker system for genotyping, population structure assessment, varietal identification, association mapping etc. (Kalia *et al.*, 2011). Paliwal *et al.*; 2016, generated microsatellite markers in *T. cordifolida* with the help of SSR enriched genomic libraries. The genomic libraries of (CT)₁₄, (GT)₁₂, (AC)₁₀, and (AAC)₈ repeats were developed, which were used to generate 90 microsatellite sequences. These g- SSR markers were validated and used for genetic diversity studies in 26 accessions of *T. cordifolia* and one each accession of *T. sinensis* and *T. rumphii*. The markers were found efficient for genetic diversity analysis as well as cross transferability of more than 80% SSR markers was also reported in related species of *Tinospora* (*T. rumphii*, and *T. sinensis*). Kumar *et al.*, 2020, developed SSR markers using SSR genomic libraries enrichment in *A. paniculata* and validated through genetic diversity analysis. Four

types of SSR enriched genomic libraries such as (CT)₁₄, (AG)₁₅, (GT)₁₂, and (AAC)₈ were used to generate 67 genomic SSR markers. The 41 SSR markers were found polymorphic and efficient for genetic diversity analysis. The developed genomic SSR markers could be an important genomic resource for crop improvement programs of *A. paniculata*. Singh *et al.*, 2014, reported genetic diversity and cross genera SSR transferability in *Vetiveria zizanioides* L. Nash by transferring rice hyper variable SSRs markers (HvSSR), out of 120 HvSSR markers studied, 36 showed cross genera transferability. The across genera transferred SSR markers of rice could be an important genomic resource vetiver germplasm improvement programme.

2.2 *EST-SSR and Transcription Factor Generation through Transcriptome*

In the last ten years, RNA-seq has emerged as the preferred platform for transcriptome analysis and has been widely used to obtain mass sequence data for gene discovery, generation of molecular markers, and transcriptional analysis in a variety of plants. Researchers can analyse functional genes and regulatory mechanisms of medicinal and aromatic plants with the aid of transcriptomics research, which can also help them refine breeding selection and cultivation methods. The transcriptome data can be used to monitor the transcriptional activity of any plant species without reference genome. Singh *et al.*, 2016, generated transcriptome sequence of *T. cordifolia* using 454 GS-FLX pyrosequencing. Identified 4,538 transcripts showing significant similarity with corresponding orthologs were categorized into 58 different transcription factor families. The highest member (457) of basic loop helix (bHLH) transcription family was identified, followed by MYB (295) and NAC (280). Among the assembled transcripts, 5,412 SSR loci consisting of mono- to hexa- nucleotide repeats and also complex motif were identified. A total of 96 EST-SSR were validated and used for genetic diversity analysis among 24 accessions of *T. cordifolia*, which indicated these markers were polymorphic and highly reproducible and can be utilized as important genomic resource.

2.3 *Genome Wide SSR Marker Generation through Whole Genome Sequencing*

Whole genome sequencing and its de novo assembly could be another approach for the generation of genomic resources in non-model plants. In case of *Bunium*

persicum whole genome sequencing was done using Illumina HiSeq X Ten sequencer. Since no reference genome was available therefore de novo assembly was done. A total of 1,77,029 perfect and 5915 compound SSR motifs were identified in 2,12,585 assembled sequences (Bansal *et al.*, 2022). Total 88 SSR primers were used for their validation and genetic diversity analysis among 25 accessions of *B. persicum*. The genome wide SSRs markers developed in *B. persicum*

will open new avenues for characterizing genotypes and to develop future conservation strategies for *B. persicum*.

The above three approaches have been used by different researchers for the generation of genomic resources in different medicinal and aromatic plants. A comprehensive information about availability of genomic resources in different medicinal and aromatic plants has been summarized in Table 1.

Table 1: The available genomic resources in medicinal and aromatic plants

S. No.	Medicinal plant species	Available genomic resources	References
1	<i>Aconitum carmichaelii</i>	Transcriptome	(Rai <i>et al.</i> , 2017b)
2	<i>Andrographis paniculata</i>	Genome, Transcriptome, g-SSRs (67), EST-SSR (32,341), NAC Transcription factors (2), WRKY Transcription Factor (58)	(Cherukupalli <i>et al.</i> , 2016; Wang <i>et al.</i> , 2017; Kumar <i>et al.</i> , 2020; Zhang <i>et al.</i> , 2021; Kumar <i>et al.</i> , 2022)
3	<i>Artemisia annua</i>	Genome, ESR-SSR (2110), NAC Transcription factor (28)	(Wang <i>et al.</i> 2012; Shen <i>et al.</i> , 2018; Kumar <i>et al.</i> , 2021)
4	<i>Bacopa monnieri</i>	Transcriptome, MYB35	(Jeena <i>et al.</i> , 2017, 2021)
5	<i>Bunium persicum</i>	g-SSRs (177029)	(Bansal <i>et al.</i> , 2022)
6	<i>Bupleurum chinense</i>	g-SSRs (19), EST-SSRs (44)	(Sui <i>et al.</i> , 2009)
7	<i>Camptotheca acuminata</i>	Genome, transcriptome	(Sun <i>et al.</i> , 2011; Zhao <i>et al.</i> 2017)
8	<i>Cannabis sativa</i>	Genome, transcriptome	(Bakel <i>et al.</i> , 2011)
9	<i>Catharanthus roseus</i>	Genome, EST-SSRs (2034), genomic-SSR (314)	(Mishra <i>et al.</i> , 2011; Shokeen <i>et al.</i> , 2011; Kellner <i>et al.</i> , 2015)
10	<i>Chrysanthemum morifolium</i>	EST-SSR (218)	(Feng <i>et al.</i> , 2016)
11	<i>Docynia delavayi</i>	EST-SSR (18)	(Peng <i>et al.</i> 2021)
12	<i>Glycyrrhiza uralensis</i>	Genome, Transcriptome, EST-SSR (7032),	(Liu <i>et al.</i> , 2015; Mochida <i>et al.</i> , 2016)
13	<i>Hippophae rhamnoides</i>	EST-SSR (30)	(Jain <i>et al.</i> , 2010)
14	<i>Lancea tibetica</i>	g-SSR (4441)	(Tian <i>et al.</i> , 2016)
15	<i>Lonicera japonica</i>	Transcriptome	(Rai <i>et al.</i> , 2017a)
16	<i>Nicotiana tabacum</i>	Genome, g-SSRs (1365), EST-SSRs (3521) NAC Transcription factor (280)	(Sierro <i>et al.</i> 2014; Tong <i>et al.</i> , 2012; Kumar <i>et al.</i> , 2021)
17	<i>Ocimum tenuiflorum</i>	Genome, ESR-SSR (471) NAC Transcription factors (110)	(Upadhyay <i>et al.</i> , 2015; Kumar <i>et al.</i> , 2021)
18	<i>Ophiorrhiza pumila</i>	Transcriptome, WRKY transcription factor (46)	(Yamazaki <i>et al.</i> , 2013; Wang <i>et al.</i> , 2022a)
19	<i>Paeonia suffruticosa</i>	EST-SSR (4,373)	(Wu <i>et al.</i> , 2014)
20	<i>Panax ginseng</i>	Genome, Transcriptome	(Li <i>et al.</i> , 2013; Xu <i>et al.</i> , 2017)
21	<i>Panax japonicus</i>	Transcriptome	(Rai <i>et al.</i> , 2016b)
22	<i>Papaver somniferum</i>	Genome, Transcriptome, EST-SSR (14957)	(Desgagné-Penix <i>et al.</i> , 2010; Winzer <i>et al.</i> , 2012; Şelale <i>et al.</i> , 2013; Pei <i>et al.</i> , 2021)
23	<i>Perilla frutescens</i>	Transcriptome	(Fukushima <i>et al.</i> , 2015)
24	<i>Physalis alkekengi</i>	Transcriptome	(Fukushima <i>et al.</i> , 2016)
25	<i>Pueraria lobata</i>	Transcriptome, g-SSR (20)	(Han <i>et al.</i> , 2015; Zhou <i>et al.</i> , 2019)
26	<i>Sarcandra glabra</i>	EST-SSR (25,620), SNP (726,476)	(Xu <i>et al.</i> , 2021)
27	<i>Swertia japonica</i>	Transcriptome	(Rai <i>et al.</i> , 2016a)
28	<i>Tinospora cordifolia</i>	genomic-SSR (90), EST-SSR (25406)	(Paliwal <i>et al.</i> , 2016; Singh <i>et al.</i> , 2016)
29	<i>Trachyspermum ammi</i>	Transcriptome, NAC Transcription factor (68)	(Howyzeh <i>et al.</i> , 2018; Kumar <i>et al.</i> , 2021)
30	<i>Trifolium pratense</i>	Genome, NAC Transcription factor (97)	(Vega <i>et al.</i> 2015; Chao <i>et al.</i> , 2018; Kumar <i>et al.</i> , 2021)
31	<i>Veratrum baillonii</i>	Transcriptome, EST-SSR (40885)	(Wang <i>et al.</i> , 2015)
32	<i>Vetiveria zizanioides</i>	Transcriptome	(Chakrabarty <i>et al.</i> , 2015)
33	<i>Withania somnifera</i>	Transcriptome, EST-SSR (729), AP2/ERF (187)	(Gupta <i>et al.</i> , 2013; Tripathi <i>et al.</i> , 2020)
34	<i>Zingiber officinale</i>	EST-SSR (16,790)	(Vidya <i>et al.</i> , 2021)

2.4 Medicinal Plants Database

Database is a collection of data that is organized for simple access, management, and updating. The genomic resource generated from transcriptome studies were uploaded for public use in the form of user-friendly database. Two medicinal plant genomic resource databases developed by ICAR-National Bureau of

Plant Genetic Resources (NBPGR), New Delhi, one is TinoTranscriptDB and another is ApTransDB. TinoTranscriptDB (<http://www.nbpgr.ernet.in:8080/Tinospora/>) and ApTransDB (<http://www.nbpgr.ernet.in:8080/Andrographis/About.aspx>), are publicly available database of transcripts and SSRs of *T. cordifolia*, and *A. paniculata*, respectively (Fig. 2). Both the database

TinoTranscriptDB
Tinospora cordifolia Transcripts & SSR Database

ICAR NBPGR

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Tinospora cordifolia (Willd.) Miers. (Giloee) plant is well known for its anti-spasmodic, anti-inflammatory, antipyretic properties and is being used in the treatment of gout, jaundice and rheumatism. *Tinospora cordifolia* is distributed throughout tropical Indian subcontinent and China. It is a shrub with large, glabrous, deciduous structure belongs to the family Menispermaceae.

The public databases and literature survey revealed very limited information with regard to genomic resources essential for judicious exploitation of *Tinospora cordifolia*. To this end, we have generated its entire transcript based database incorporating information of gene sequences and gene-based SSR (EST-SSR) or microsatellite markers. SSR marker is very useful tool because of its co-dominant inheritance, multi-allelic variation and high reproducibility. Since EST-SSRs markers, designed in this database is derived from transcript sequences, it can have various applications in the field of genomics as well as understanding functional diversity of allelic variants, if any. Moreover, it is equally useful for the generation of high density marker based genetic maps for gene/QTL discovery, phylogenetic studies and comparative genomics.

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ApTransDB
Andrographis Transcripts & SSR Database

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Andrographis paniculata is a herb well known in Asia and belongs to the family *Acanthaceae*. It is native to Asia and distributed throughout India, China, Sri Lanka and Taiwan. *Andrographis paniculata* is a potential medicinal plant of immense economic importance. Leaves of *A. paniculata* contains active constituents like andrographolide, homoandrographolide, andrographestrol and andrographonone. *A. paniculata*, also known as *Kalmegh* in India, is mentioned in ancient Indian medicine (Ayurvedic) literature as a constituent of ayurvedic formulation used for the treatment of liver disorder, and neoplasm. The plant has also hepatoprotective, antioxidant, and hypoglycemic activities and is being used in the management of immune system and neoplastic diseases.

Due to limited information with regard to genomic resources, essential for exploitation of *Andrographis paniculata*, we have generated a database from its entire transcriptome including information relevant to gene sequences and gene-based SSR (EST-SSR) or microsatellite markers. SSR marker is an indispensable tool for molecular mapping, diversity analysis and phylogenetic studies. Moreover, since SSR were designed from transcripts sequences, it is equally useful for comparative genomics including candidate gene determination. Besides molecular markers, the sequences of transcripts will serves as an important resource for the discovery of biosynthetic genes and other regulons associated with its active constituents.

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Fig. 2. Database of microsatellite markers generated from transcriptome *Tinospora cordifolia* (Tino TranscriptDB) and *Andrographis paniculata* (Andro TranscriptDB)

Table 2. Genomic resource databases of medicinal and aromatic plants.

S.No.	Name of database	Description	URL
1	TinoTranscriptDB	Transcripts and SSR database of <i>Tinospora cordifolia</i>	http://www.nbpr.ernet.in:8080/Tinospora/
2	ApTransDB	Transcripts and SSR database of <i>Andrographis paniculata</i>	http://www.nbpr.ernet.in:8080/Andrographis/About.aspx
3	croFGD	Catharanthus roseus functional genomics database	http://bioinformatics.cau.edu.cn/croFGD/
4	TCMPG	Integrative database for traditional Chinese medicine plant genome	http://cbcb.cdutcm.edu.cn/TCMPG/
5	MPGR	Medicinal plant genomic resources database	http://mpgr.uga.edu/

provides the information of SSR, (EST Expressed sequence tags)-SSR, transcription factor categories, and GO categories, and gene sequences. The genomic information provided can be further utilized to discover the candidate genes related to secondary metabolite biosynthesis through comparative genomics. The different public databases available in case of medicinal and aromatic plants are given in Table 2.

3. Conclusions

Genomic resources such as molecular markers, genes, and transcription factors related to the biosynthesis of bioactive compounds or secondary metabolites are important tools that can be utilized for increasing the production of these compounds. Since very limited genomic resources has been generated in case of medicinal plants, therefore there is need to develop more resources so that, obstacles for crop improvement programs of medicinal and aromatic plants can be addressed effectively.

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