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 Transciptome, 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., antioxidant, 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-inflammatory, anti-angiogenic, hepato-renal protective, and insecticidal activities (Okhuarobo 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 perfume, and cosmetic industries (Sethi et al., 1968). Bunium persicum (Boiss.) Fedtsch., commonly known as Kala
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.](image)

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 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. cordifolida 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 Table1.

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

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


Paliwal R, R Kumar, DR Choudhury, AK Singh, S Kumar, A Kumar, KC Bhatt, R Singh, AK Mahato, NK Singh, R
Singh (2016) Development of genomic simple sequence repeats (g-SSR) markers in 

Pei L, B Wang, J Ye, X Hu, L Fu, K Li, Z Ni, Z Wang, Y Wei, L Shi, Y Zhang (2021) Genome and transcriptome of Papaver somniferum Chinese landrace CHM indicates that massive genome expansion contributes to high benzylisoquinoline 


Rai A, H Kamochi, H Suzuki, M Nakamura, H Takahashi, T Hatada, K Saito, M Yamazaki (2017a). De novo transcriptome assembly and characterization of nine tissues of Lonicera japonica to identify potential candidate genes involved in 

sequencing and de novo transcriptome assembly of Swertia japonica to identify genes involved in 

Rai A, M Yamazaki, H Takahashi, M Nakamura, M Kojoma, H 
Suzuki, K Saito (2016b). RNA-seq transcriptome analysis of Panax japonicus, and its comparison with other Panax 
species to identify potential genes involved in the saponins biosynthesis. Front. Plant Sci. 7.481

Sequencing and Expression Analysis of Aconitum carmichaelii to Analyze Key Genes Involved in the Biosynthesis of 

Şelale H, I Çelik, V Gültekin, J Allmer, S Doğanlar, A Frary 
(2013) Development of EST-SSR markers for diversity and 

Sethi KL, ML Maheswari, VK Srivastava, R Gupta (1968) 
Natural variability in Vetiveria zizanioides collection from 

Shen Q, L Zhang, Z Liao, S Wang, T Yan, PU Shi, M Liu, X 
Fu, Q Pan, Y Wang, Z Lv (2018). The Genome of Artemisia annua Provides Insight into the Evolution of Asteraceae 

Shokeen B, S Choudhary, NK Sethy, S Bhatia (2011) Development of SSR and gene-targeted markers for construction of a 

Sierro N, JN Battey, S Ouadi, N Bakaher, L Bovet, A Willig, 
S Goepfert, MC Petisch, NV Ivanov (2014) The tobacco genome sequence and its comparison with those of tomato 

Singh R, R Kumar, AK Mahato, R Paliwal, AK Singh, S Kumar, 
SS Marla, A Kumar, NK Singh (2016) De novo transcriptome 

Singh R, D Narzary, J Bhardwaj, AK Singh, S Kumar, A Kumar (2014). Molecular diversity and SSR transferability studies in 

Chemistry and medicinal properties of Tinospora cordifolia (Guduchi). Indian J. Pharmacol. 35(2):83.


Sui C, J Wei, S Chen, H Chen, C Yang (2009) Development of 
6233–6240.

Sun W, L Leng, Q Yin, M Xu, M Huang, Z Xu, Y Zhang, 
H Yao, C Wang, C Xiong, S Chen (2019) The genome 
of the medicinal plant Andrographis paniculata provides 
insight into the biosynthesis of the bioactive diterpenoid 

Sun Y, H Luo, Y Li, C Sun, J Song, Y Niu, Y Zhu, L Dong, A 
Lv (2011). Pyrosequencing of the Camptotheca acuminate 
transcriptome reveals putative genes involved in camptothecin 

Tian Z, F Zhang, H Liu, Q Gao, S Chen (2016) Development of 
SSR Markers for a Tibetan Medicinal Plant, Lancea tibetica (Phrymaceae), Based on RAD Sequencing . Appl. 

Tong Z, Z Yang, X Chen, F Jiao, X Li, X Wu, Y Gao, B Xiao, W Wu (2012) Large-scale development of 
microsatellite markers in Nicotiana tabacum and construction of a genetic 

Tripathi S, Y Srivastava, RS Sangwan, NS Sangwan (2020) In 
silico mining and functional analysis of AP2/ERF gene in 

Upadhyay AK, AR Chacko, A Gandhimathi, P Ghosh, K Harini, 
AP Joseph, AG Joshi, SD Karpe, S Kaushik, N Kuravadi, 
CS Lingu (2015) Genome sequencing of herb Tulsi (Ocimum 
tenuiflorum) unravels key genes behind its strong medicinal 

Vega JJ, S Alying, M Hegarty, D Kudrna, JL Goicoechea, A 
Ergon, OA Rognli, C Jones, M Swain, R Geurts, C Lang 
(2015) Red clover (Trifolium pratense L.) draft genome 

Vidya V, D Prasath, M Snigdha, R Gobu, C Sona, CS Matti (2021) 
Development of EST-SSR markers based on transcriptome 
and its validation in ginger (Zingiber officinale Rosc.). PLoS 
ONE 16(10):e0259146.


