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Conversion of Metabolomic Data to Genomic Marker for Genetic Characterization of *Piper betle* L. Chemotypes: A Review

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ABSTRACT

The Indian perfumery industry is shifting towards natural product. In India including West Bengal betel leaves produces high quality essential oil as well contribute to Indian fresh vegetable export. The crop is cultivated from stem cutting and suffers from authenticity problem of cultivars with redundant names. The genetic screening and characterization of cultivars were not initiated due to unavailability of reliable markers. The essential oil metabolomic study identified some polar and non-polar volatile signature compounds. Metabolomic profiling of cultivars is not consistent due to seasonal variation in the production of secondary metabolites and ignorance in marking of unique trace discriminatory compounds. In this paper gene ontogeny study was made on major signature compounds to obtain the complete coding sequence (CDS) of the aroma-genes. The CDS information of aroma-genes could be utilized to construct robust DNA markers to eradicate authentication problem and germplasm management of *Piper*. The direct genomic analysis could supersede the metabolome profiling. Information available in NCBI, DDBJ and EMBL database were searched for gene ontogeny study utilizing available metabolomic data. The information and method depicted could be efficiently utilized for *Piper* genomics. Aroma-scientists could apply this technique to validate promising cultivars and competent germplasm management.

Key words: Aroma-gene ontogeny, Chemotypes, Discriminatory signature compound, Essential oil, Genome, Metabolome, *Piper betle*, Transcriptome.

The ancient art of perfumery was a generous gift of the plant kingdom. Perfume was associated with a paying of respect and homage from time immemorial. Ayurveda, the world's oldest healing system endures the essential oil application in health treatments. The sense of smell was not given so much priority during early civilization but with the course of cultural evolution the society gave significant value to the faculty of fragrance leading to the development of a whole new branch of manufacturing industry of perfumery. The fragrance industry depended on the horticulturist who determined the best time to pluck the fragrant plant part and the process remained a mystery in the period of alchemy (Septimus, 2018).

The fragrance specialists even noted that the flowers yield perfumes in all climates but the summer perfumes were more prolific in odour while the winter ones were the sweetest. Along with latitudinal difference seasonal variation were also noticed by the manufacturers. From ancient alchemist to modern chemical perfumer possess the knowledge that seasonal variation and geographical location commands the delicacy of the fragrance. A ubiquitous understanding was prevalent from time antiquity that a perfectly volatile oil owe to the fragrance of the plant during its life from blossom (Pietra, 2002).

Essential oils are volatile substances physically isolated from odoriferous plants. The volatile liquid present in essential oil are predominantly insoluble in water and freely soluble in alcohol, vegetable oil and mineral oil (Sharma, 2014). The variation in fragrance in different seasons ensure epigenetic control of the trait as well as environmental influence. The multi-faceted function of the essential oil complicates its ontogeny. Essential oil act as attractant and aid in natural selection, protect against parasites, act as protective shield in plant injury (Fokou *et al*., 2020).

The advent of distillation procedure and new solvents revolutionized the perfume industry. In present century the cosmetic industry is shifting towards the natural aroma that was replaced by synthetics in the middle time frame with progress in organic chemistry. Medicinal and Aromatic plants (MAP) are drawing marked interest in the current scenario with varied application in medicine, phyto-pharmacy, phyto-toxicology, cosmetics and food industries (Zouari, 2013). Our own subcontinent possess a number of MAP that has immense potential to be utilized in the perfume industry.

Piper betle L. belonging to Piperaceae family produces good quality essential oil. This heart-shaped leaf is intensely

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linked to the cultural life of the sub-continent of India. Betel leaf is an important horticultural crop with tremendous export value. State of West Bengal occupies a prominent position in production of this cash crop. 'Bangla', 'Mitha', 'Sanchi' and 'Benarasi' are the major varieties grown in West Bengal. Betel leaves occupy high medicinal as well therapeutic values. The landraces were different from each other in the composition of essential oil and shows difference in metabolomic profiling but genetic characterization of this aromatic crop is still incomplete (Mondal *et al*., 2020).

The betel farmers address their cultivars with different vernacular names. The lack of authentication and use of numerous synonym creates problem in marketing and branding of promising landraces especially during export of oil as well as fresh leaf. Bioactivity based studies in betel is restricted to local races and a composite inventory is not available to screen elite germplasm on the basis of bioactive components (Makkar *et al*., 2017). The selection of germplasm depending on phyto-constituents could aid faster cultivar identification and need based industrial application (Das *et al*., 2018).

Essential oil chemotyping could act as reliable source in geographic identification of cultivars, decrease in redundancy problem, ambiguity in nomenclature and increase in taxonomic fidelity in *Piper*. Studies reveal the presence of several aromatic volatile compounds in different parts of betel plants, such as leaves, inflorescence, rhizome, flower parts with noticeable qualitative and quantitative variation. These compounds are regarded as signature chemicals. Bio-tagging of the signature compounds with genetic markers could assist in the amelioration of the intraspecific taxonomic complexity of *Piper betle* cultivars.

In this paper an attempt is taken to correlate the available biomarking information of important secondary metabolites found in the *Piper betle* essential oil for effective utilization to construct DNA based reliable markers and application of the same for confirmation of distinct landraces and cultivars.

In this paper a data-mining was done on the available information on biomarkers including all the signature compounds of *Piper betle*. 150 papers were initially screened on metabolomics of *Piper* with a search tab using Piper + metabolome in Google Scholar. The abstracts of the screened papers were examined and 23 out of them were found to provide the details of signature compounds of betel leaves and its rhizomes. In this study emphasis was given to rare compounds to tag cultivars. The extensive study of metabolomic data were utilized for searching of aroma-genes of *Piper* using NCBI BLAST method, DDJB, EMBL database and GENECARDS. The data on complete coding sequence (CDS) was obtained for the selected biomarkers contextual to Indian *Piper* cultivars. In this study the genetic information of ortholog of related aroma-plants were also taken into account were direct sequence data was lacking for betel. Additional 5 papers related to functional genomics were incorporated in this study to recognize future scope of this kind of research. The information generated in this study could be efficiently used for genomic marking of *Piper*. The method included study of available data on genetic markers, essential oil profile and signature compounds present in *Piper betle*.

Unavailability of genetic marker in betel cultivar

In Indian subcontinent the betel leaves are grown by vegetative propagation leading to anomaly in true identification of landraces and cultivars. The stem cutting with 2-3 leaves were used as new propagating material. The continuous process of phenotypic selection by farmers reduces the number of evaluated clones leading to narrowing of genetic base diversity. The vegetative mode of propagation led to the vast difference in composition of secondary metabolites in *Piper betle* in India. Age old traditional cultivation technique in *Piper* could generate epigenetic trans-generational variation leading to transformation of an established clone into a debased performer. In such a condition clonal performance analysis through an inclusive process of multi-trait evaluation, multi-staged selection and genetic parameter estimation could authenticate cultivar fidelity (Bisognin, 2011). Authentication is a fundamental part in quality assessment of herbal medicines and in modern era performed using several novel techniques. Authentication method could assess the exact herbal formulation of a cultivar leading to bio-marking of plants. The 'Bangla' variety of *Piper* has numerous synonym and variously known as 'Godi Bangla', 'Simurali Bangla', 'Calcutta Bangla', 'Sada Bangla', 'Kali Bangla', 'Ramtek Bangla', 'Desi Bangla', 'Dasi paan', 'Bangla Dashi' and so on in different parts of India with anonymous identity (Jaiswal *et al*., 2014). Such a spectrum of local nomenclature requires validation through a reliable method.

In betel vine the genetic screening of different cultivars and landraces were possible but no concrete molecular marker is available, that is specific to individual chemotypes. RAPD (Ranade *et al*., 2002) and SCAR (Samantaray *et al*., 2012) markers are available for sex determination in betel but effort was not given in the identification of reliable marker for germplasm screening and cultivar identification. In only one molecular study with ISSR markers involving 15 cultivars including 4 'Bangla' variants showed 'Bangla Kalkattiya' and 'Mahoba Bangla' occupying same cluster and two 'Bangla' cultivars showing close proximity in dendrogram. The Jacquard's similarity index and Principle componenet analysis (PCA) was used to estimate the relatedness of cultivars. The two 'Bangla' cultivars showed a similarity index of 0.72 but they were not totally identical. The ISSR based DNA fingerprinting could be applied in samples with nomenclature ambiguity but the inclusion of more reliable marker could validate differential cultivar performance (Goyat, *et al*., 2016) as well as medicinal and pharmacological acceptance of *Piper* cultivars.

In recent era integrated approaches that combine metabolome, transcriptome and proteome profiling have gained prudence and have proven to generate novel insights

in the understanding of the biological pathways leading to generation of concrete markers (Liu *et al*., 2018). A previous knowledge of the plant phenotype and field performance may aid in first approach to interpret complex omics knowledge and consequent visualization of the data on any template (Rosato *et al*., 2018). Molecular marker based characterization of the genotypes is a reliable tool for selection, management and authentication of perennial horticultural cash crops. DNA-based marking could reduce redundancy of similar cultivars and selection of geographically suitable cultivars.

In *Piper betle* mostly the quality assessment was made on the basis of leaf constituent. The study adopting multi-dimensional-TLC-fingerprinting could detect authentic and counterfeit medical formulation. The HCA-TLC analysis was able to differentiate inter-specific variation but the research was insufficient to comprehend varietal profiling. Though the mode of the study was purely related to pharmaceutical authenticity of the components depending on PCA and HCA profiling. The method was not able to differentiate *Piper nigrum* from *Piper betle* cluster and was not strong enough to differentiate cultivars under *Piper betle* species (Wirasuta *et al*., 2016). Though the TLC-densitometric study of different commercial varieties and heterogeneous relative frequency (hRf) value based discrimination could be applied to assess the level of cultivar complexity.

Application of consistent and reliable marker such as SSR, EST-SSR were not reported for germplasm management in betel-vine (Patra *et al*., 2016) To eliminate synonym problem, firm authentication, elite germplasm identification and chemotypic confirmation at molecular level, the available metabolome of *Piper* leaves may play a significant role. Although a lot of work was conducted regarding the identification of non-polar and polar compounds present in the betel-vine essential oil but no study was conducted so far to correlate the metabolomic information with available gene ontogeny to construct robust DNA markers for authentication of the cultivars and landraces.

Essential oil based bio-marking

The variation in essential oil of *Piper betel* plant may show qualitative and quantitative difference in the amount of phytochemicals grown in different geographic region. The yield of essential oil depends upon genetic factor, soil, climate and management factors. The composition and quality of oil depends on age of plant, organ/ tissue age, time of harvesting, inter-cultural practices, method of extraction, process parameters, duration of extraction, nature of solvent, pretreatment, time of storage before extraction. The yield of essential oil of betel leaf varies from 0.09% to 0.80% on fresh weight basis (Periyanayagam, 2012). The world's highest yield and finest quality leaves are produced in East and West Midnapore districts of West Bengal province of India (Guha, 2007).

The oil yield of commercial varieties in betel extractor for 2.5 hr including 'Tamluk Mitha', 'Ramnagar Mitha', 'Kali Bangla', 'Sada Bangla', 'Sanchi' shows yield of 2.0%, 2.0%, 1.7%, 1.7%, 0.8% respectively on dry weight basis (Guha, 2007). Studies indicate 'Mitha' variety as highest essential oil producer compared to others though several synonym exist for this variety as 'Mitha cum bangla', 'Mitha Calcutta', 'Tamluk Mitha', 'Ramnagar Mitha' (Basak and Guha, 2015). In another result including 13 cultivar maintained in Andhra Padesh showed 'Gaach paan', 'Mitha Calcutta', 'Godi Bangla', 'Mitha cum Bangla', 'Maghi', 'Karapaku' with promising oil yield of 0.31%, 0.51%, 0.47% and 0.33%, 0.39%, 0.23% respectively than 'Tellaku of Utukur variety' (0.09%), 'Tellaku of Punnur' (0.13%), 'Tellaku of Chenmur' (0.11%) 'Pachaikodi' (0.20%), 'Kariele' (0.18%), 'Bangla' (0.20%) varieties (Guha and Nandi, 2019). The above studies clearly indicate the superior quantitative performance of East-Indian cultivars than their Southern counterparts.

Screening of seven varieties from different locations of West Bengal identified 45 components. The total constituents were subdivided into 14 monoterpenes, 23 sesquiterpenes, 8 phenyl propenes. In 'Bangla', 'Bagerhati', 'Manikdanga', 'Ghanagate' varieties, the prominent components were eugenol acetate (31.46-43.97%) and eugenol (13.13-33.06%), whereas in 'Mitha' variety it was chavicol (23.85%), in 'Chhaanchi', safrole (42.77%) was the chief ingredient (Karak *et al*., 2018). In the promising 'Mitha' variety if large proportion of anethole remains absent, the sweet fragrance may still exist due to presence of estragole with similar organo-leptic properties (Basak and Guha, 2015). In South Indian cultivars high level of safrole is present in 'nadan' and 'kuzhikkodi' cultivar while high percentage of eugenol with trace safrole was detected in 'salen' variety. The cultivars vary significantly from each other on the basis of qualitative and quantitative variation of essential oil (Muhammed, 2007). In *Piper betle* the quantitative profiling of the presence of essential oil may take a pivotal role in cultivar identification.

Identification of signature compound

In *Piper betle* the chemical polymorphism is well established leading to formation of eight distinct chemotypes predominantly on the basis of essential oil present in leaves. Additionally the rhizome oil may form another significant biomarker (Thanh, 1997). The available metabolomic data could be used to identify the transcriptomics and genetic ontogeny of volatile compounds. The identification of genes and their sequence data could be utilized for the construction of robust, reliable, co-dominant DNA-primers for the germplasm screening research. Bioactivity based screening of betel vine is restricted to local landraces and integration of the regional data was incomplete though available. The categorization of major compounds as metabolic marker is not complete and shows overlapping of chemotypes. In this study it is being observed that in metabolic profiling and plants systematic more emphasis is given on the percentage of essential constituents though the categorization based on unique, trace compounds could be more accurate. Trace

elements could play a significant role in phylogenetic classification as discriminatory component. A careful investigation of the metabolomic data may identify signature compound for efficient bio marking of chemotypes as well as phyto-toxicological analysis.

Some intrinsic and extrinsic compounds regulates the heterogeneous mixture of secondary metabolites found in essential oil of MAPs (Zouari, 2013). The interplay of genetic and epigenetic factors leads to variation in the transcriptome of different chemotypes on the basis of differential gene expression. Metabolomic profiling and chemometric studies of eight local varieties of West Bengal on the basis of polar and non-polar metabolites showed 'Mitha' and 'Chhaanchi' chemotypes were different from the other chemotypes of Bengal. Metabolomic study distinctly differentiated 'Chhaanchi' chemotype from others by the presence of safrole and sabinene. The identified metabolites showed their presence irrespective of season. 'Mitha' variety differentiated due to presence of chavicol, chavicol acetate and estragole (Karak *et al*., 2016).

Seasonal variation in accumulation of metabolites

In a study involving Gas Chromatography-Mass Spectroscopy (GC-MS) of leaf extract on the accumulation of various metabolites in *Piper*, it was detected that winter shows the maximum storage of chemical constituents gradually decreasing towards monsoon. Cluster analysis of the 8 varieties on the basis of 45 non-polar components revealed seasonal variation of components. In summer principle component analysis (PCA) showed formation of distinct cluster where 'Mitha' and 'Chhaanchi' comes under one cluster and 'Bangla', 'Bagerhati' and 'Haldi' formed another cluster with rest of the varieties 'Manikdanga', 'Ghanagate' and 'Kalibangla' occupying the last one. The same varieties performed differently in monsoon. 'Chhaanchi' differentiated from rest of the varieties in metabolite profile. 'Manikdanga'-'Mitha' formed close association segregating from others keeping in another cluster. In winter also 'Mitha' and 'Chhaanchi' differed from other varieties but the 'Mitha' variety showed at least 5 unique signature components and 9 metabolites in excess to 'Chhaanchi' and others. 'Bagerhati', 'Kalibangla' and 'Ghanagate' formed second cluster with rest of the varieties are kept in one (Karak *et al*. 2016). Chemometric profiling distinctly showed wide qualitative and quantitative difference of polar and non-polar compounds in respect to seasonal cycle. The study necessitates the inclusion of Partial least square discriminant analysis (PLS-DA) over PCA (Mohottalage *et al*., 2007). An extensive, repetitive study on all polar and non-polar compounds with focus on compounds with steady accumulation irrespective of season will be more dependable and authentic. Table 1 shows the chemotypic identification of different cultivars of *Piper betle.*

Conversion of metabolomic data to gene ontogeny

In functional genomics the forward movement from DNA sequences to biological function may become complex due the unavailability of suitable markers. The systematic analysis of gene function could be assayed using transcriptome, proteome or metabolome. Scientists used principal component analysis and partial least square discriminant analysis to show change in relative amount of ten volatile constituents in essential oil content of *Piper betel* with respect to season (Karak *et al*., 2018). The discriminatory unique compounds could be used in molecular biomarking and more precisely for construction of authentic primers for varietal identification. Inspection on eugenol synthase gene associated with eugenol acetate production is potentially important for biomarkering of essential oil for its omnipotent presence in fragrant plants taking from rose flower to betel leaf (Saxena *et al*. 2014 and Yan *et al*., 2018).

Majority of the Piper betle scientists estimated nine different volatile constituents from different chemotypes to be applied as biomarkers. Though seasonal variation of eugenol and sesquiterpenoids were noticed during GC-MS analysis but their unambiguous presence in *Piper* is important in primer or probe construction. Chavicol, isoeugenol, eugenol, germacrene D, safrole, anethole, chavibetol, eugenol acetate, alpha-cadinol were reported to be the major component of essential oil (Satyal and Setzer, 2012). The varietal assessment through aroma components were insufficient to aid efficient varietal profiling and vine management and justifies the urgency to generate heritable molecular markers.

Proposition to generate robust DNA markers

As genomic markers are rare in *Piper betle,* the application of metabolomic data may give impetus in generation of

Table 1: Taxonomic identity of *Piper betle* Cultivars on the basis of highest percentage of volatile chemical present in leaf essential oil.

Volatile Biomarker	Chemotype
Chavicol	Sagar Bangla (48%), Mitha (23.85%)
Isoeugenol	Vietnameze (72%)
Eugenol	Kapoori (33.22%), Bangla (63.56%), Selan (58.0%), Sanchi (25.90%)
Germacrene D	Sirugamani (16.07%)
Safrole	Desawari (45.34%), Sanchi (22.75%), Kuzhikkodi (35.60%), Sri Lankan (52.70%), Taiwanese (28.0%)
Anethole	Mitha (19.30%)
Chavibetol	Phillipine (53.10%), Malaysian (69.0%), Nepalese (80.50%)
Eugenol acetate	Kali Bangla (22.16%), Manikdanga (44.03%), Bangla (35.77%), Ghanagete (43.97%), Bagerhati (31.46%)
Alpha-cadinol	Vietnameze betel rhizome

heritable DNA marking system. Ten prominent signature compounds identified from the metabolomic data were utilized for NCBI blast study to comprehend about the linear DNA or RNA sequence. Additionally DDJB, EMBL, GENECARDS databank was also searched for information on these aroma genes related to bio-markers identified in *Piper betle*. The gene accessions from the same genus or related fragrant genomes were studied including ortholog. The extensive study revealed the complete coding sequence (CDS) for each signature compound.

The CDS data sequence may be effectively utilized for generation of reliable primers such as SSR, ISSR, SNP other than universal RAPD primers. The ortholog gene sequences available from other aromatic plants with same volatile compounds may be used as initial information for gene sequence identification. The gene ID could be directly used for constructing primers in NCBI portal. The available genetic data of MAPs could be utilized for the study of complete gene ontogeny. A large number of primers could be constructed and applied for validation in *Piper betle* genetic characterization using NCBI primer BLAST or by using Primer 3, Primer 3 plus free tools. Table 2 exhibits the gene ontogeny of common metabolite compounds found in *Piper betle*.

In functional genomics this reverse route from metabolome to genome may prove useful and strongly assist in molecular taxonomy and advanced breeding operations. The development of user-friendly AMBAB web-server for genomic research and breeding of asparagus (*Vigna unguiculata)* is an excellent example were the available dry-lab information was used to enrich molecular breeding. The system integrated multiple analytical tools. Blast, Sim4, Primer-3, e-PCR were conglomerated to access available genomic resources for plant geneticists, breeders and advanced farmers. The systematic use of reference genome sequences help the users in primer construction and prediction of amplicon patterns (Luo, *et al*. 2015). These type of advance forecasting becomes productive in setting of experimental design, reduction in cost and aid in time management of the breeder. This kind of web-server could be developed for aromatic and medicinal plants (MAP) incorporating local data for sequence analysis and marker development, advancing MAP-genomics in under-developed countries.

In *Piper* the intra-varietal genetic variation was not well characterized but some incomplete data is available with metabolite profiling. Intra-varietal crop performance could significantly regulate stress response, differential local performance, specific retaliation to evolving pest and diseases. Transcriptome sequencing allows genome wide analysis of diverse plant genomes to identify biologically significant SNPs. In barley deep sequencing and analysis of unigenes identified 'Baudin' and 'Gairdner' varieties. Significant SNPs represented 9.65% in 'Baudin' and 14.64% in 'Gairdner' genetic variation (Rani and Sharma, 2017). SNP variation could effectively determine varietal difference and

Table 2: Gene Ontogeny of common metabolite marker compound of *Piper betle*.

Biomarker	Gene	Transcriptome	Gene ID	Chromosome	Type
Chavicol	CVOMT1	Chavicol-o-methyltransferase	LOC105167564 NCBI	LG8	Protein Coding
Isoeugenol	IGS1	Isoeugenolsynthase	DQ372813 DDBJ,ENA	Q15GI3	972 cds,m-RNA
Eugenol	RcEGS1	Eugenol synthase	JQ522949 DDBJ, ENA	M1FGX5	1137 cds,m-RNA
Germacrene	GDS	Germacrene D synthase	LOC112170354	Chr 6	4261 linear mRNA
Safrole					
Anethole	TAO	Trans-anethole oxidase	H8Y0R3, ENA	HQ889281	1047, cds. Genomic DNA
Chavibetol/	EGS1	Eugenol synthase	DQ372812 ENA	Q15G14	945 bp cds, mRNA
Estragole	OMT1	trans-resveratrol di-O-methyltransferase	LOC103426312 NCBI	MJAX01000014	3395, cds, gene
Sabinene	TPS24	Beta-myrcene/(E)-beta-ocimene synthase 2	822173, AT3G25810	AT3G25810	4211, cds. gene
Alpha-cadinol	CAD1_A	Delta cadinene synthase isozyme A	108463032ncbiQ43714 ENA	X96429	555 bp cds

required to be incorporated in *Piper* genome management. In aromatic world the utilization of reference unigene data of allied MAP plants producing same secondary metabolites could be an efficiency measure leading to smart agriculture.

In another intensive study involving sesame (*Sesamum indicum*) transcriptome profiling, genic region specific marker discovery identified 4 DEGs and 379 SSRs. The application of bio-analytical tools additionally marked several SNPs and INDELs (Jaiswal *et al*., 2020). The study of cellular metabolic pathway along with signaling response and conglomeration of DEG database information in singularity proved valuable in crop improvement program were DNA-based genomic research was not sufficient. In *Piper* the genetic study is inadequate and an innovative approach similar to DEG bio-tool could widen the scope of incorporation of bioinformatics knowledge of related crops.

In a proteomic approach genes for pod distortion syndrome (PDS) was identified in soyabean. A comparison of protein expression profile of PDS and non-PDS cultivars detected candidate proteins related to the growth retardation syndrome. nESI-LCMS/MS based proteomic analysis confirmed expression of 3 proteins in PDS cultivars. Nascent polypeptide associated complex alpha subunit, Rubisco large subunit and oxygen evolving enhancer protein 2 were the key regulator in green stay phenotype and PDS in soyabean, This CDS information of associated proteins could be converted into genomic primer or probe construction through simple bioinformatics tool for resistant cultivar identification (Payghamzadeh *et al*. 2017).

The genetic study in aromatic plant system is menial and some researchers tried to integrate metabolome, transcriptome and genome. In *Valeriana jatamansi* SNP polymorphism and GC-MS analysis of volatile compounds were used to differentiate the wild type from the common-garden samples. The volatile compounds distinctly marked specific chemotypes and were validated using discriminatory compounds. This study provided information about an initial attempt to use both metabolomic and genomic data in separation of wild and commercial aroma-plants (He *et al*., 2018).

Rao and his group categorized *Murraya koenigii* (L.) Spreng into 14 chemotypes under 3 major categories (monoterpenoid, sesquiterpenoid and mono- and sesquiterpenoid predominant oils) based on leaf essential oil profiles. The monoterpenoid plants were grouped into 7 chemotypes. The sesquiterpenoid and the mono- and sesquiterpenoid plants were divided into 3 and 4 chemotypes, respectively (Rao *et al*., 2016). The chemotypic clustering of plants on the basis of essential oil offers opportunity for the consumers and flavourist to select additional trait apart from the conventional yield and vigour. This study exemplifies the application of metabolome information in commercial food sector.

The gene expression pattern of terpenoid biosynthesis was studied in *Cinnamomum camphora* by transcriptome analysis. RNA sequencing identified 67 candidate unigenes involved in terpenoid biosynthesis. The identified genes showed correlation with protein of two precursor pathways of terpenoid. The upregulation of *TPS*-gene series were observed in borneol types than linalool chemotypes. The bioinformatics analysis discovered genes involved in terpene synthesis and their precursor in MEP (2-C-methyl-D-erythritol 4-phosphate) and MVA (Mevalonate acid) pathways. Multiple unigenes were annotated with the same enzyme as they represent various alternatively spliced transcript or member of the same gene family (Gang *et al*., 2002 and Chen *et al*., 2018). These aromatic gene expression studies contain potential to be used in *Piper* bioinformatics studies.

In *Cymbopogon martini* of Poaceae family by high-through-put transcriptome analysis of terpenoid biosynthetic pathway, 913 unigenes were identified related to 287 metabolic pathways. The inflorescence tissue validated the presence of two monoterpenes, geranyl acetate and geraniol (Koeduka *et al*., 2009). The up-regulation of six genes encoding alcohol dehydrogenase, geranyldiphosphate synthase, fernasyldiphosphate synthase, aldehyde dehydrogenase, aldo-keto reductase and alcohol acyltransferase expression patterns were studies using qRT-PCR. A transcriptome database analysis revealed the presence of 60 transcription factors in addition to 6 up-regulated proteins. Transcriptome analysis of *C. martinii* inflorescence could be used for understanding of the molecular mechanisms of essential oil biosynthesis in the genus *Cymbopogon* and related plant families (Kaur *et al*., 2019).

Piper betle is a widely cultivated plant of India with high commercial value but it lacks genomic information and molecular research. West Bengal exports huge amount of *Piper* leaf to different parts of world earning significant foreign revenues but the varietal admixture, redundancy of local names were main constraint in the export and sell of pure produce. The essential oil and secondary metabolites were of immense value in medicine and aroma industry. The metabolome profiling of *Piper* exhibited some aromatic compounds also present in other MAP species. The signature compounds present in *Piper* could be used for construction of DNA-based robust, reliable, heritable markers for varietal screening. The functional genomics resource of other MAP plants offer opportunity to be applied in *Piper* genomic research. The utilization of functional genomics and dry-lab bioinformatics tool could advance the *Piper* cultivation, production, reduction in redundancy problem, vine management, industrial utilization and export authentication.

CONCLUSION

Aromatic essential oil has large applications in fragrance, perfumery, cosmetics and pharmaceutical industry. Owing to the tremendous applications of the essential oil from the genus *Piper*, knowledge of the biochemical and molecular regulatory processes and expressions of genes involved in

terpenoid biosynthesis pathway, is highly desirable to increase the extraction of essential oil. The progress of perfumery industry as well as raw leaf export get acceleration if the *Piper betle* chemotypic identification becomes complete. The authenticity of the cultivars and landraces could facilitate and satisfy the consumer need as well as export of this high-value cash crop. As metabolomic marking is not consistent and influenced by seasonal variation, heritable genetic marking is required for cultivar authentication.

This investigation more over emphasizes the prospect of effective utilization of trace elements in validation study by acting as discriminatory compound. The available gene ontogeny data in *Piper* is inadequate and this study may be utilized for construction of novel DNA markers. If a vast array of primers could be generated, they may assist in inception of heritable biomarking program of *Piper betle* plants and conservation and patenting of betel cultivars. The present study may assist the aroma-scientists and horticulturist in molecular diagnosis of essential oil of *Piper betle* plants leading to complete the gap in genomic study.

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