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Review Article

MOLECULAR REVIEW: *SESAMUM* EMERGING AS A DROUGHT TOLERANT OLDEST OIL CROP

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ABSTRACT
Sesamum is one of the oldest oil crop traditionally and medicinally used since ages and act as a substitute for cooking oil. Sesame is gaining popularity for its antioxidant derivatives, numerous health benefits and for economically very useful seed oil. Sesame oil with 85% unsaturated fatty acids, is highly stable with reducing effect on cholesterol levels and prevents coronary heart diseases among other several health benefits. Being short duration crop sesame grows in all seasons in a year, fits well into various cropping systems and emerging as an important drought tolerant oil crop. The objectives of the review were to emphasize on the work carried out mainly on molecular grounds in *Sesamum indicum*. Different molecular markers are used across Sesame accessions in wild and elite germplasms to study the genetic diversity across the world. Average to high polymorphism is detected with different molecular markers. Apart from genetic diversity studies, gene and association mapping work is also reviewed. High-density genetic linkage map and QTL(Quantitative Trait Loci) analysis for sesame provides a good foundation for further research on sesame genetics and for marker-assisted selection.

Keywords: *Sesamum indicum, Genetic diversity, QTLs, Mapping*

INTRODUCTION

Sesame is an annual herb reaching an height of more than one meter (Mina Kazemian Ruhi *et al*., 2014) and belongs to the family Pedaliaceae. *Sesamum indicum* L. syn *S. orientale* L. is cultivated species whereas *S. malabaricum* or *S.mulayanum* are wild species apart from *S. calycinum*, sp Baumii. Other specices, *S. angustifolium*, and *S.radiatum* are also used as food (Elly Kafiriti and Omari Mponda, 2008). *Sesamum*, queen of oil seeds, originated in Africa (Ethiopia) spread through West Asia, China, Japan and thought to be first domesticated in Africa (Nayar and Mehra, 2008) and India(Bedigian, 2004). Around 60 to 65 countries all over the world produce sesame seed, distributed across Asia, Africa, Europe, Central and South America of tropical and temperate regions. Among them Asia and Africa are the major sesame producing countries. The world on an average produces close to 3 million tons of sesame seed every year. Major sesame producing countries are India, China, Myanmar, Sudan, Pakistan, Mexico, Ethiopia, Sri Lanka and Burma whereas Sudan and Nigeria are major exporting countries. According to NMOOP 2015-16 reports, India covers an area (lakh ha) of 18.93 under cultivation, followed by Myanmar (11.11), Nigeria (5.29), China (4.53), Ethiopia (3.20), Ugand (2.1) and other countries(34.1). China produces the highest yield of 1234 kg/ha seed and India an average yield of 413 kg/ ha. In India, West Bengal produces maximum yield when compared to other sesame producing states for its many nutraceuticals, industrial and pharmaceutical uses(Bradley Morris, 2002). High amounts of both sesamin and sesamolin compounds are present (Sirato-Yasumoto *et al*., 2001) in sesame which increases the hepatic mitochondrial and peroxisomal fatty acid oxidation rate. It also contains antioxidant and health promoting activities (Kato *et al*., 1998), apart from preventing cancer and heart diseases

(Cooney *et al*., 2001). Keeping in view its increasing importance's an attempt has been made to review *Sesamum* Genetic Diversity, QTL analysis and Gene Mapping methods.

GENETIC DIVERSITY WITH DIFFERENT MOLECULAR MARKERS

Plant genetic diversity can be conserved through modern analytical tools and techniques which are widely available for genetic manipulations. Genomic diversity studies plays important role in determining the evolution of traits. Different biological approaches were reviewed by Sharad Tiwari *et al*., 2011 to overcome the slow pace of improvement in sesame crop through tissue culture, molecular marker-assisted breeding, functional genomics and genetic transformational studies. Similar study was also reviewed by Ekta Sharma *et al*., in 2014 on genetic divergence in sesame emphasizing on different molecular markers usage. A over view of genetic diversity was assessed with molecular markers is given in Table:I. Komivi Dossa *et al*., 2016 studied the genetic diversity of 96 sesamum accessions collected from 22 countries distributed over six geographic regions of Africa and Asia were genotyped using 33 polymorphic SSR markers revealing large genetic variability within the germplasm. Similarly Rapheal *et al.,* 2018 identified more than fifty percent of SSR markers which are polymorphic in accessions collected in Northern Ghana region. A detail study was reviewed by Komivi Dossa *et al.,* 2017 regarding the genetic resources so far available.

Sovetgul *et al*., 2018 studied 129 sesame landraces and cultivars using 70 simple sequence repeats (SSR) markers which resulted in 23 polymorphic markers which produced 157 alleles. The number of alleles arranged form 3-14. In a recent study in assessing the genetic diversity by Eveline de Sousa Araujo *et al.,* 2019 used ten SSR markers to assess 36 sesame germplasms which showed average polymorphism. Polymorphism was also obtained by Anandan *et al*., 2017 when assessed among low diversified sesame accessions. Nweke Friday Nwalo, 2015 observed genetic diversity in Nigerian Sesame cultivars and its relationship with phytochemical composition using SSR markers and with spectrophotometric methods. High genetic variability was observed among the genotypes with significant variations in phytochemical composition. Similar study was also carried by Jong-Hyun Park *et al*., 2013 in germplasm collected from different parts of the world with SSR markers. Vijaya Sudhakara Rao Kola *et al*., 2012 used specific microsatellite markers, of which forty six markers found to be polymorphic with genetic similarity coefficient ranging from 79 to 92% suggesting that the cultivars developed are from diverse origin exhibiting good variability. Kiranmayi *et al*., 2016 and Raphael Adu-Gyamfi *et al*., 2019 concluded the same by working with SSR markers.

Whereas fairly low genetic diversity was observed in Turkish accessions when EST based SSRs were used (Ummu Seyitalioglu, 2010), whereas higher heterozygosity obtained when Daniel Endale Gebremichael and Heiko Parzies, 2010 used ten Simple Sequence Repeats (SSRs) markers to study genetic variation in Ethiopian accessions. In a study carried out by Anupam dixit *et al*., 2005 fifty microsatellite sequences were isolated from an enriched library of sesame, out of which ten polymorphic microsatellites were used to determine the diversity. When Maini Bhattacharjee *et al.,* 2019 assessed 30 diverse genotypes for genetic diversity with SSRs, showed PIC 0.87. Whereas Labhya Rani Gogoi *et al.,* 2018 when assessed 33 indigenous genotpyes of sesame with 27 polymorphic SSR markers resulted in an average of 2.87 allele per locus with polymorphism information content (PIC) value varied from 0.99 to 0.01. Aejaz Ahmad Dar *et al.,* 2017 studied germplams collected from different agroclimatic zones of India and assessed with 22 RAPD and 18 SSR primers showing high genetic variability. Even in few germplams assessed for genetic diversity showed polymorphism. Khames Mourad *et al.,* 2019 evaluated few germplasm assessed with only 5 SSR markers showed 53% polymorphism. Through employing selective hybridization strategy, 95 mining expressed sequence tags from the NCBI database used to characterize genetic diversity of 16 sesame germplasms by Jyothi Badri *et al.,* 2014. The number of alleles per microsatellite locus ranged from 2 to 5 with an average of 3.11 alleles.

Apart from SSRs, genetic diversity was also observed with other molecular markers such as ISSR (Admas Alemu *et al.,* 2013; Hitesh Kumar *et al*., 2012), EST-SSR (Sarita Pandey *et al*., 2015 and Arna

Das *et al*., 2013), RAPD (Fazal Akbar *et al.*, 2011) and AFLP (Hernan Laurentin *et al.*, 2008; Ghulam Ali, 2007). Dagmawi Teshome *et al*., 2015 studied 82 sesame accessions from Ethiopia and 38 exotic germplasms with 6 ISSR primers which resulted in high polymorphism among the local germplasms. Similar work was also carried out by Mohammed Abate *et al*., 2015 with 128 accessions with 7 ISSR primers gave 92.2% polymorphism. Admas Alemu *et al.*, 2013 studied the genetic diversity of sesame in major growing areas of Ethiopia. Six farmers cultivars and varieties of sesame from the north western Ethiopia each consisting of ten individual samples were analyzed using four inter simple sequence repeat (ISSR) markers. The four ISSR primers yielded 37 amplification products of which 36 bands exhibited polymorphism. ISSR markers were also used by Tapaswini Hota *et al*., 2016 to study the genetic relationship and agro-morphological characters in sesame. Out of the tested 30 ISSR primers, 18 primers produced 114 detectable fragments of which 97(85.08%) were polymorphic.

Sesame accessions collected from Sudan were assessed for genetic diversity with RAPD markers showed high polymorphism (Abdellatef *et al*., 2008). Twelve Cambodia and Vietnam sesame accessions were evaluated for genetic diversity with RAPD markers by Toan Duc Pham *et al*., 2011 revealed relatively high genetic diversity. Similarly Satyendra Nath, 2014 studied with 200 RAPD markers to determine the genetic diversity among 60 Indian sesame genotypes. Germplasm collected from different geographical regions of the world were also analyzed for genetic diversity by Rajendra Pujar and Chandrashekar Patil, 2016. They studies 40 sesame germplasms screened RAPD markers and obtained 122 polymorphic fragments. High genetic diversity was also obtained by Soumen Saha *et al*., 2019 when 15 sesame germplasm from West Bengal were analyzed for genetic diversity with RAPD markers. Patil *et al*., 2016 also worked with RAPD markers to determine the polymorphism among the sesame accessions. Sarita Pandey *et al*., 2015 confirmed that both genetic and phenotypic diversity in a combined way could efficiently evaluate the variations present by studying 60 genotypes with 36 microsatellite markers.

Ghulam Ali, 2007 assessed 96 sesame accessions collected from different parts for the world using AFLP technique. Twenty one primers generated a total of 445 bands and among them 157 were polymorphic. Florent Jean-Baptiste Quenum and Qichuan Yan, 2017 assessed genetic variation among a mini core germplasm of sesame using biochemical and RAPD markers. A SDS-PAGE of protein extracts of sesame seeds revealed 31 protein markers, out of which only 4 were polymorphic indicated that this technique is also suitable for studying genetic diversity. Mediterranean sesame accessions were analyzed for genetic diversity with 5292 high quality SNPs identified by double-digest restriction site associated DNA (dd RAD) sequencing by Merve Basak *et al*., 2019. Results indicated a highly dense genetic resource among the collection and with a genetic distance between pairs of accessions 0.023 to 0.524.

Table I: Over view of the genetic diversity assessment with different molecular marker systems in *Sesamum indicum*

S.No.	Author	Collection	Accessions	Marker Type	Marker Number	Result
1	Anupam dixit *et al*., 2005	Korean	16	SSR	50	PIC ranged from 0.34 to 0.80
2	Ghulam Ali 2007	Different parts of the world	96	AFLP	21	65% polymorphic
3	Abdellatef E *et al.*, 2008	Sudan	10	RAPD	25	Low level of similarity
4	Hernan Laurentin *et al.*, 2008	Different parts of the world	10	AFLP	8	Traits affected by chemical phenotype
5	Ummu Seyitalioglu 2010	Turkish	161	EST-SSR	318	Low Genetic Diversity
6	Daniel Endale Gebremichael and	Ethiopian	50	SSR	10	PIC ranged from 0.393 to 0.820

	Heiko K Parzies 2010					
7	Fazal Akbar *et al.*, 2011	Pakistan	20	RAPD	10	75% polymorphic
8	Toan Duc Pham *et al.*, 2011	Cambodia and Vietnam	12	RAPD	10	Highly polymorphic
9	Sharad Tiwari *et al.*, 2011	Different parts of the world	96	AFLP	21	35% polymorphic
10	Vijaya Sudhakar Rao Kola 2012	India	9	SSR	207	Good Variability
11	Hitesh Kumar *et al.*, 2012	Punjab, India	94	ISSR	34	Overlapping diversity
12	Admas Alemu *et al.*, 2013	Ethiopia and North western region	6	ISSR	4	High genetic diversity
13	Arna Das et ala 2013	Indian genotypes	7	EST-SSR	4	Genetic diversity value above 0.5 between parents
14	Jong-Hyun Park *et al.*, 2014	Korea and China	70	SSR	14	PIC=0.23to 0.77
15	Satyendra Nath Sharma 2014	Indian genotypes	60	RAPD	10	High polymorphism
16	Nweke Friday Nwalo, 2015	Nigerian	30	SSR	10	High genetic variability
17	Sarita K Pandey et ala 2015	Different parts of the world	60	EST-SSR	36	High Diversity
18	Mohammed Abate *et al.*, 2015	Ethiopia	128	ISSR	7	92.2% polymorphic
19	Admas Alemu *et al.*, 2015	Farmer cultivars and north western Ethiopia	6	ISSR	4	High genetic diversity
20	Anne Frary *et al.*, 2015	Turkish	137	AFLP	140	Low Variability
21	Kiranmayi SL *et al.*, 2015	Andhra Pradesh, India	23	SSR	10	4 markers polymorphic
22	Komivi Dossa *et al.*, 2016	22 countries	96	SSR	33	Large genetic variability
23	Tapaswini Hota *et al.*, 2016	Different geographical regions of the India	33	ISSR	30	85.08% polymorphic
24	Kiranmayi *et al.*, 2016	Local area of Andhra Pradesh, India	23	SSR	10	4 % polymorphic
25	Rajendra B Pujar and Chandrashekar Patil G 2016	Dharwad, India	40	RAPD	28	High level of polymorphism

26	Patil CG *et al.*, 2016	Dharwad, India	7	RAPD	28	Average Polymorphism
27	Anandan R *et al.*, 2017	Tamil Nadu, India	9	RAPD and SSR	10	Appropriate for evaluation of low diversified sesame varieties
28	Bazel H Ali Al somain *et al.*, 2017	Saudi and from different geographical environment	52	SRAP	17	High Degree of genetic polymorphism
29	Florent Jean-Baptiste Quenum and Qichuan Yan 2017	China	15 Mini core germrplasm	RAPD	53	High Genetic Similarity
30	Sovetgul *et al.*, 2018	Korea	129	SSR	70	High variance individuals within populations
31	Labhya Rani Gogoi *et al.*, 2018	Jorhat, India	33 Indigenous genotypes	SSR	27	High diversity among local germplasm, PIC=0.43
32	Rapheal *et al.*, 2019	Northerrn Ghana	25 land races	SSR	38	Highly polymorphic
33	Eveline de Sousa Araujo *et al.*, 2019	Brazil	36	SSR	10	Significant genetic diversity
34	Souman Saha *et al.*, 2019	West Bengal, India	15	RAPD	25	High Genetic diversity
35	Maini Bhattacharjee *et al.*, 2019	Different parts of West Bengal, parts of India and USA and Bulgaria	30	SSR	32	PIC=0.87

IDENTIFICATION OF QTLS AND LINKAGE MAPS

Restriction Fragment Length Polymorphism is considered as the first DNA-based molecular marker system and it is used for the preparation of linkage maps and for mapping of several traits of interest in many crops. The greater abundance and other desirable features of RFLPs as compared to phenotype and protein markers, prompted the development of other relatively more convenient DNA marker systems like random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), etc. Single nucleotide polymorphism (SNP) has emerged as the most abundant molecular marker that is amenable to high-throughput genotyping. RAPD method detects high level of polymorphism in plants and used to construct high-density genetic maps in several crop species(Singh and Singh, 2015).

Haiyang Zhang *et al.*, 2013 were the first to study QTL with high density linkage map in Sesamum. Two accessions coI1134(white seeded, P1) and R x BS (black seeded, P2) were taken for segregation, three replicates of the P_1, P_2, F_1, BC1, BC2 and F2 populations were grown. An F_2 population of 260 is taken to construct a genetic linkage map and locate QTLs. Estimated size of the sesame genome is 1380.938cM. A total of 653 marker loci were identified on 14 linkage groups. The dominant heritability for all QTLs

ranged from 1.28-7.18%. A significant negative correlation in oil content with that of protein content were found through association mapping among 369 world-wide sesame germplasm accessions under five environments using 112 polymorphic SSR markers when used by Chun Li *et al.,* 2014 (Table II)

Kun Wu *et al*., 2014 identified a total of 3,769 SNPs from RAD-Seq using 89 polymorphic PCR markers including 44 Expressed Sequence Tag- simple sequence repeats(EST-SSRs), 10 genomic SSRs and 35 Insetion-Deletion Markers. The final map resulted in 1,230 markers distributed on 14 linkage groups. While Venkata Ramana Rao *et al*., 2014 developed a molecular map for important agro-botanic traits in sesame. Two genotypes, Chandana and TAC-89-309 were studied for F_2 population, F_1 and Parents were evaluated under field conditions for nine agro-botanic traits. Based on parental polymorphism(23.07%), a mapping population of 120 F_2 individual with 60 RAPD markers were selected to map nine linkage groups, and about 60% of the genome with length ranging between 58.8 to 423.8cM resulted in identification of ninteen QTL and two genomic regions. Out of the 19 QTLs identified, one QTL for corolla colour, number of nodes were detected.

Yanxin Zhang *et al*., 2013 constructed a high density genetic map containing 201,488,285 pair-end reads. Total of 71,793 high quality SLAFs were detected of which 3,673 SLAFs were polymorphic and 1,272 of the polymorphic markers met the requirement of use in the construction of a genetic map. Lin Bin *et al*., 2009 used three types of PCR-based markers to construct a map for F2 segregating population of an intraspecific cross between two cultivars of sesame. Ten EST-SSR markers, 80 AFLP markers and 244 RSAMPL markers were screened to obtain 284 polymorphic loci. A total of 220 molecular markers were mapped in 30 linkage groups with a genetic length of 936.72cM. Haiyang Zhang *et al*., 2013 studied sesame cp(chloroplast) genome which is a circular molecule containing a total of 153,338 base pairs and a total of 114 unique genes. Similar study was carried out by Dong-Keun Yi and Ki-Joong Kim (2012) on complete chloroplast (cp) genome of *S. indicum*. The length is almost similar with slight variation of 153,324bp and has a pair of inverted repeat (IR) regions that comprise 25,141bp each. The two IR regions divide the genome into a large single copy (LSC) region and a small single copy (SSC) region. The LSC region is 85,170bp, whereas the SSC region is 17,872bp. The complete cp sequence of *S.indicum* is 153,324bp in length of which 58% is coding regions and 42% is non-coding regions.

In recent studies, genome-wide SNP makers were used for assessing genetic diversity structure and linkage disequilibrium by Chengqi Cui *et al*., 2017. Genotyping of 366 sesame germplasm accessions by using 89,924 high quality SNPs. All these SNPs covered all 13 linkage groups. Largest number of SNPs were found on LG5 followed by LG3 and least number occurred on LG4 with lowest marker density on LG6. Further studies by Haiyang Zhang *et al*. 2016 in determining the growth habit indicated that sesame recessive gene controls the determinate trait dt1 and a second determinate line, dt2. Later, Hua Du *et al*. 2019 constructed a genetic map which compromised of 2159 SNP markers distributed on 13 linkage groups and was 2128.51cM in length with an average distance of 0.99 cM between adjacent markers.

Linhai Wang *et al*., 2017 through his study, developed 7,357 SSR markers from sesame genome and transcriptomes and a genetic map was constructed by generating 424 novel polymorphic markers using cross pollination with 548 recombinant inbred lines. The genetic map developed with 13 linkage groups, ranged in size of 113.6 to 179.9cM and 14 QTL for sesame Charcoal rot disease resistance. Similar approach was carried out by Ayse Ozgur Uncu *et al*., 2016 studied through sequencing analysis resulted in a total of 15,521 SNPs. Ramya and Bhat, 2018 developed 60 SSR markers used in genomic hybridization assay, of which 12 were found to be polymorphic. The linkage map constructed contained 26 markers in three linkage groups covering a distance of 298.6cM, 694.4cM and 86.4cM. Similar study was also carried out by Xin Wei *et al*., 2014 in genetic linkage map construction and marker assisted selective breeding in sesame to identify a total of 218 SSR markers through whole genome survey.

Genes for morphological as well as biological traits were also studied to construct high resolution genetic maps. Hongxian Mei, 2017 constructed a genetic map with 9,378 SLAF markers with 13 linkage groups. The map spanned a total genome of 1,974.23 cM and the mean LG length of 151.86 cM. Genes for basal branching habit and flowers per leaf axils were mapped to LG5 and LG11 respectively. Haiyang Zhang;

Hongmei Miao *et al*., 2013 analyzed genetic segregation and quantitative trait loci(QTL) for sesame seed coat in 6 generations. Results showed that two major genes with additive-dominant epistatic effects and polygene with additive-dominant epistatic effects were responsible for controlling seed coat color trait. Rong Zhou *et al*., 2018 studied genome-wide association of 39 sesame seed yield related traits. They observed a total of 646 loci were significantly associated with the 39 traits and resolved to 547 quantitative trait loci(QTLs). Genome wide association study was carried out by Komivi Dossa *et al*., 2019 for traits related to drought tolerance using 400 diverse sesame accessions, including landraces and modern cultivars revealed ten stable QTLs explaining more than 40% of the phenotypic variation and four linkage groups were significantly associated with drought tolerance related traits.

Table II: Genetic and Association mapping studies in *Sesamum indicum*

S.No.	Author	Trait/genome assembly	Marker Type/method	Population studied	Result
1	Lin-Bin *et al.*, 2009	Linkage map	EST-SSR, AFLP, RSAMPL	F2 segregation	Genome length 1,232.53 cM
2	Dong-Keun Yi and Ki-Joong Kim 2012	Chloroplast genome	Cp DNA sequence analysis	------	Chloroplast genome size 153,324 bp in length
3	Yanxin Zhang *et al.*, 2013	High density genetic map	SLAF-seq	F2 population	Final map length 1,474.87cM
4	Haiyang Zhang, *et al.*, 2013	Seed coat colour	AFLP and RSAMPL	P1, P2, F1, BC1, BC2,and F2	Two major genes for seed coat colour
5	Haiyang Zhang *et al.*, 2013	Chloroplast genome	Illumina technology	-----	Chloroplast genome size 153,338 bp in length
6	Chun *et al.*, 2014	Seed oil and protein content	SSR	369 germplasm accessions	A negative correlation to oil content to protein content
7	Venkata Ramana Rao *et al.*, 2014	Agro-botanic traits	60 RAPD	120 F2	19 QTLs identified
8	Kun Wu *et al.*, 2014	Grain traits	RAD-seq	RIL	13 QTL identified
9	Ayse Qzgur Uncu *et al.*, 2016	------	Genotyping by Sequencing approach	RIL population	15,521 SNPs identified
10	Haiyang Zhang 2016	Growth habit	Re-sequencing	Parents and F2 population	Same recessive gene controls the dt1 and dt2
11	Linhai Wang *et al.*, 2016	Plant height and seed coat colour	Genome assembly	430 recombinant inbred lines	41 QTLs for plant height and 9 for seed coat colour
12	Chengqui Cui *et al.*, 2017	Growth habit	Genome wide SNP	366 sesame accessions	LG5, LG3 largest SNPs identified
13	Linhai Wang *et al.*, 2017	Charcoal rot resistance	SSR	548 recombinant inbred lines	14 QTL for charcoal rot disease resistance

14	Hongxian Mei *et al.*, 2017	Basal branching & flower per leaf axil	SLAF-seq	300 population of BC1	Genetic distance 1,974.23cM
15	Ramya P an Bhat KV 2018	Yield traits	SSR	Hydridization Assay	Three linkage groups
16	Rong Zhou *et al.*, 2018	39 seed yield related	Genome wide association studies	705 diverse lines	82 QTLs and candidate genes, SiLPT3 and SiACS8
17	Hua Du *et al.*, 2019	Seed related	SLAF-seq	F2 population	Genetic map 2128.51 cM in length
18	Komivi Dossa *et al.*, 2019	Drought tolerance	Genome wide association studies	400 diverse accessions	Ten stable QTLs identified

CONCLUSION

Due to increasing demand for edible oils with the growing population, there is substantial need to increase cultivation of oil crops. Among the different oil crops cultivated, *Sesamum indicum* cultivation is increasing on a larger scale, and there is a need for extensive research in obtaining better varieties which are high in yield traits. So far minimum molecular studies were carried out. Still there is a need for further studies and its confirmation. And more important, there is a need to stabilize the results obtained and further carry out extensive studies which improvises the yielding aspects by balancing abiotic and biotic factors through molecular techniques in *Sesamum indicum*

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