DUS CHARACTERIZATION AND MOLECULAR DIVERSITY ANALYSIS IN SESAME (*Sesamum indicum* L.)

BY

AMIDALA MANASA

B.Sc. (Hons.) Ag.

THESIS SUBMITTED TO THE
ACHARYA N.G. RANGA AGRICULTURAL UNIVERSITY
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF

MASTER OF SCIENCE IN AGRICULTURE

(GENETICS AND PLANT BREEDING)

CHAIRPERSON: Dr. D. BHARATHI

DEPARTMENT OF GENETICS AND PLANT BREEDING
SRI VENKATESWARA AGRICULTURAL COLLEGE, TIRUPATI
ACHARYA N.G. RANGA AGRICULTURAL UNIVERSITY
GUNTUR- 522 034, A.P.

2023

DECLARATION

I, **Ms. AMIDALA MANASA,** hereby declare that the thesis entitled **"DUS CHARACTERIZATION AND MOLECULAR DIVERSITY ANALYSIS IN SESAME (*Sesasum indicum* L.)"** submitted to the **Acharya N.G. Ranga Agricultural University** for the degree of **Master of Science in Agriculture** is the result of original research work done by me. I also declare that no material contained in the thesis has been published earlier in any manner.

Place: TIRUPATI
Date:

(AMIDALA MANASA)
I.D. No. : TAM/2021-027

CERTIFICATE

Ms. AMIDALA MANASA has satisfactorily prosecuted the course of research and that thesis entitled **"DUS CHARACTERIZATION AND MOLECULAR DIVERSITY ANALYSIS IN SESAME (*Sesamum indicum* L.)"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that neither the thesis nor its part thereof has been previously submitted by her for a degree of any University.

Place: TIRUPATI
Date:

(Dr. D. BHARATHI)
Chairperson
Senior Scientist
Dept. of Genetics and Plant Breeding
Regional Agricultural Research Station
Tirupati- 517 502, A.P.

CERTIFICATE

This is to certify that the thesis entitled **"DUS CHARACTERIZATION AND MOLECULAR DIVERSITY ANALYSIS IN SESAME (*Sesamum indicum* L.)"** submitted in partial fulfilment of the requirements for the degree of **'MASTER OF SCIENCE IN AGRICULTURE'** of the Acharya N. G. Ranga Agricultural University, Guntur is a record of the bonafide original research work carried out by **Ms. AMIDALA MANASA** under our guidance and supervision.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part and all assistance received during the course of the investigations have been duly acknowledged by the author of the thesis.

Thesis approved by the Student's Advisory Committee

Chairperson	:	**Dr. D. BHARATHI** Senior Scientist Dept. of Genetics and Plant Breeding Regional Agricultural Research Station Tirupati - 517 502, A.P.	_____
Member	:	**Dr. M. SHANTHI PRIYA** Professor Dept. of Genetics and Plant Breeding S.V. Agricultural College Tirupati - 517502, A.P.	_____
Member	:	**Dr. P. LAVANYA KUMARI** Assistant Professor and Head Dept. of Statistics and Computer Applications S.V. Agricultural College Tirupati - 517502, A.P.	_____

Date of final viva-voce:

ACKNOWLEDGEMENTS

It is by the grace of Almighty, omnipotent blessings of God Lord Sri Venkateswara I could accomplish and bring to light this humble piece of work for which I am externally indebted.

First of all, I owe an immeasurable debt of gratitude to the person who made the biggest difference in my life, my mentor and chairman of advisory committee ***Dr. D. Bharathi,*** *Senior Scientist, Department of Genetics and Plant Breeding, Regional Agricultural Research Station, Tirupati. My diction of thanks will be incommensurate for her perspicuous nature, impeccable advice, untiring help, caring attitude and constructive suggestions which have instilled in me the script of confidence to successfully complete the task for which I will be grateful for the rest of my life. I cannot but consider myself luck to have worked under her guidance.*

I am very much pleased to place my appreciation and gratitude to the member of my advisory committee ***Dr. M. Shanthi Priya,*** *Professor, Department of Genetics and Plant Breeding, S.V. Agricultural College, Tirupati for her amiable suggestions, benevolent and noble ideas and kind cooperation during pursuit of my research work.*

I am extremely thankful to ***Dr. P. Lavanya Kumari,*** *Assistant Professor and Head, Department of Statistics and Computer Applications, S.V. Agricultural College, Tirupati, andmember of my Advisory Committee for her encouragement, valuable suggestions, amenable and stupendous guidance during the course of investigation.*

I am truly indebted and thankful to ***Dr. V. L. N. Reddy,*** *Associate Professor and Head, Department of Molecular Biology and Biotechnology, S.V. Agricultural College, Tirupati for critical evaluation of my research work giving attention to every intrigues with valuable guidance, constant encouragement, constructive and timely advice, inestimable patience and utmost care taken amidst his busy schedule throughout the course of this study.*

I am extremely thankful to ***Dr. M. Reddi sekhar****, Professor and Head,* ***Dr. P. Shanthi,*** *Associate Professor and* ***Dr. M. Sreevalli Devi,*** *Assistant Professor****, Dr. B. Reddi Yamini****, Teaching Associate, Department of Genetics and Plant Breeding, S.V. Agricultural College, Tirupati for their encouragement, sustained interest, prompt and dedicated efforts in providing all the necessary facilities required without which the successful completion of this task would not have been possible.*

*From my mere existence on earth to present situation, my every step of life is moulded by my Mother (****Smt. A. Vijaya Bharathi****), Father (****Sri. A. Sreenivasulu****) and Brother (****A. Manoj****), I will forever remain indebted to them. Words cannot express*

their innumerable sacrifices, blessings, love, everlasting encouragement and support. Their parenting brings every time the best in every effort of my venture.

My deep and sincere gratitude to my Grandfather ***Sri. A. Sreeramulu*** *and other family members for their continuous and unparalleled love, help and support. I am forever indebted to them for giving me several opportunities and experiences.*

I express my heartful thanks to ***Mrs. Mounika*** *and* ***Mr. Asib****, Technical Assistant and other field staff and farm labour for their timely co-operation in the farm operations.*

My heartily thanks to my seniors ***Suvarna, Sravani, Manoj, Pavani, Mounika, Divya, Hema sai, Praveen, Soumya, Vineesha, Jyothirmai, Vineela*** *for constant help, motivation and support during my course study.*

My heartfelt thanks to my friends ***Bhanu, Swarna, Anil, Girish, Soumya, vidya, Venkat, Lokesh, Vishnu, Praveen, Nagendra, Sai teja, Sudheer*** *for their friendly assistance, valuable cooperation and suggestions during my research work.*

I owe a lot to my best friends ***Venkateswarlu, Sindhuja, Thanusha, Dattamma, Leela, Ishwarya*** *who inspired, helped and boosted my self-confidence.*

It is a pleasure to acknowledge the affection and help rendered by Non-teaching staff ***Venkata Lakshmi, Thyagaraju Naidu, Munirathnam and Ramesh****, Department of Genetics and Plant Breeding, S. V. Agricultural College, Tirupati.*

I am thankful to ***Acharya N. G. Ranga Agricultural University*** *for allowing me to pursue my Post Graduation successfully, for providing the facilities and financial assistance to conduct my research work.*

Finally, word of appreciation goes to ***Sri Sai Graphics****, Balaji colony, Tirupati for printing this thesis in time.*

Everyone may not have been mentioned but none is forgotten.

Manasa….✍

LIST OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

LIST OF PLATES

LIST OF APPENDICES

LIST OF SYMBOLS AND ABBREVIATIONS

$\overline{X}$	:	Grand mean
%	:	per cent
<	:	Less than
>	:	Greater than
µl	:	Microlitre
°C	:	Degree centigrade
2-D	:	2 Dimensional
∞	:	Infinity
ANOVA	:	Analysis of Variance
CD	:	Critical Difference
cm	:	Centimeter
CV	:	Coefficient of Variation
d.f	:	Degrees of freedom
DNA	:	Deoxyribose Nucleic Acid
dNTP	:	Deoxyribose Nucleotide Tri Phosphate
DUS	:	Distinctness, Uniformity, Stability
EDTA	:	Ethylene Diamine Tetraacetic Acid
et al.,	:	and other people
EtBr	:	Ethidium Bromide
etc.	:	And so forth
g	:	Gram
GA	:	Genetic Advance
GAM	:	Genetic Advance as per cent of Mean
GCV	:	Genotypic Coefficient of Variation
h^2 (bs)	:	Heritability in broad sense
HCl	:	Hydrogen Chloride
hr	:	Hour
HRM	:	High Resolution Melting
m	:	Metre
M	:	Molar
mg	:	Milligrams
min.	:	Minute
ml	:	Millilitre

mM	:	Milli molar
mm	:	Millimeter
NaCl	:	Sodium Chloride
ng	:	Nano grams
nm	:	Nanometer
NTSYS	:	Numerical Taxonomy and Multivariate Analysis System
OD	:	Optical Density
PBRs	:	Plant Breeders' Rights
PCR	:	Polymerase Chain Reaction
PCV	:	Phenotypic Coefficient of Variation
PIC	:	Polymorphism Information Content
pM	:	Pico molar
ppm	:	Parts Per Million
PPV and FR	:	Protection of Plant Varieties and Farmers' Rights
psi	:	Pounds per square inch
PVP	:	Plant Variety Protection
PVP	:	Poly Vinyl Pyrrolidone
QR	:	Quick Response
QTL	:	Quantitative Trait Loci
rpm	:	Revolutions Per Minute
RT-PCR		Real Time- Polymerase Chain Reaction
sec.	:	Second
UPGMA	:	Unweighted Pair Group Method with Arithematic Mean
UPOV	:	International Union for the Protection of New Varieties of Plants
v/v	:	Volume/volume
via	:	Through
viz.,	:	Namely
w/v	:	Weight/volume

Name of the author	:	**AMIDALA MANASA**
Title of the thesis	:	**"DUS CHARACTERIZATION AND MOLECULAR DIVERSITY ANALYSIS IN SESAME (*Sesamum indicum* L.)"**
Degree to which thesis is submitted	:	**MASTER OF SCIENCE**
Faculty	:	**AGRICULTURE**
Department	:	**GENETICS AND PLANT BREEDING**
Chairperson	:	**Dr. D. BHARATHI**
University	:	**ACHARYA N.G. RANGA AGRICULTURAL UNIVERSITY**
Year of submission	:	**2023**

ABSTRACT

The present investigation was conducted at Regional Agricultural Research Station, Tirupati during *Rabi*, 2022-23 to characterize sesame genotypes for DUS traits, to study the genetic parameters (variability, heritability and genetic advance), genetic divergence and to develop varietal-specific DNA fingerprints of sesame using molecular markers.

DUS characterization of 43 sesame genotypes using 20 DUS traits revealed the existence of substantial diversity among the characters and also appreciable differences were observed for the traits *viz.,* days to 50 % flowering, petal colour, petal hairiness, branching, branching pattern, stem hairiness, leaf lobes, leaf size, leaf serration of margin, capsule hairiness, capsule shape, capsule number per leaf axil, capsule arrangement, capsule length, days to maturity, seed colour, 1000-seed weight and oil content. These descriptors would aid in explicit the identity of genotypes.

The analysis of variance carried out among 43 genotypes for 10 yield and yield attributes revealed significant differences among the genotypes for all the characters indicating the presence of considerable amount of genetic variability in the studied material. Higher estimates of PCV and GCV were exhibited by seed yield per plant indicating ample amount of variation among the genotypes for this trait. Thus, direct selection for this trait would be rewarding for improvement of yield. High heritability in conjunction with high genetic advance as per cent of mean was observed for number of primary branches per plant, number of capsules per plant and seed yield per plant indicating the predominance of additive gene action and direct selection would be effective for improvement of these traits.

Genetic diversity studies indicated the existence of significant diversity among 43 sesame varieties and grouped them into seven clusters. Cluster II had maximum intra cluster distance while inter cluster distance was highest between cluster IV and VII followed by cluster III and IV, cluster IV and VI, cluster V and VII and cluster II and VII representing that genotypes belonging to these clusters were more divergent. Days to maturity contributed relatively maximum towards genetic divergence followed by oil content, number of primary branches per plant and days to 50 % flowering. The genotypes Paiyur-1 from cluster II, JCS-1020 from cluster VII, Nirmala from cluster IV, G.Til-4 from cluster III and Hima from cluster I could be selected as parents in future breeding programme as they expressed high *per se* performance for more number of traits, maximum inter cluster distance and complementarity for more number of traits of interest.

Molecular profiling of 43 sesame genotypes revealed that out of 34 SSR markers used, 9 were polymorphic and generated alleles ranging from 2 to 4 with an average of 2.4 per locus. Polymorphism information content (PIC) values varied from 0.045 (SSR 212) to 0.549 (SEM 12-65) with an average of 0.279. The polymorphic percentage is 26%. DNA fingerprinting with 9 polymorphic SSR markers distinguished 17 genotypes whereas, by using both 12 DUS descriptors and alleles codes of nine polymorphic markers, differentiated all the 43 sesame varieties. The QR codes were generated for all the 43 genotypes using combination of DUS traits and allele codes. Therefore, both morphological and molecular diversity are important for the identification of varieties.

In the present study, based on *per se* performance, morphological diversity using D^2 statistic and UPGMA cluster analysis, the crosses *viz.,* Paiyur-1 × Pratap, JCS-1020 × Nirmala, G.Til-4 × Pratap and Hima × Nirmala could be suggested for future breeding programme for development of high yielding sesame varieties.

Chapter - I

Introduction

Chapter-I

INTRODUCTION

Sesame (*Sesamum indicum* L., 2n = 26), a member of the Pedaliaceae family, is widely cultivated in Africa and Asia. It is labelled as the "Queen of oilseeds" because of its high oil content, delicious nutty aroma, and flavour. Sesame seed contains a considerable amount of oil, proteins, carbohydrates, essential minerals, a high amount of methionine and tryptophan, fibers as well as secondary metabolites such as lignans, saponins, flavonoids, and phenolic compounds. Moreover, the seeds are a good source of calcium, phosphorus, iron, are rich in vitamin B, E, and a small amount of trace elements. Sesame oil has an excellent stability due to natural antioxidants *i.e.,* sesamin, sesamolin, and sesamol (Vanishree *et al.,* 2022). In addition, it has excellent characteristics like relatively short growing season, high drought resistance and small diploid genome size of ~337 MB (Wang *et al.,* 2014) to ~350 MB (Wei *et al.,* 2017).

Sesame global production has reached 125 lakh hectares and 63.5 lakh tonnes production, with an annual average productivity of 508 kg/ha, while in India, it is grown in an area of 16.27 lakh hectares with the production of 7.88 lakh tonnes and with productivity 485 kg/ha (Yadav *et al*., 2022). Globally, India ranks first in area (46.5%) under sesame cultivation and stands at the second place in terms of production in world after Myanmar. The major sesame growing states in India are Gujarat, Uttar Pradesh, Andhra Pradesh, Tamil Nadu, Karnataka, West Bengal, Bihar and Assam. In Andhra Pradesh, it is cultivated in an area of 0.40 lakh hectares with the production of 0.08 lakh tonnes and with productivity 215 kg/ha (www.Indiastat.com, 2021-22). The average productivity of Andhra Pradesh is far below to the national average (485 kg/ha) as well as the world average (508 kg/ha).

Despite its prominence among oilseeds, numerous beneficial effects of sesame oil on human health, cosmetics and engineering applications, the

productivity levels of sesame is not spectacular due to its cultivation in sub marginal lands, narrow adaptability, non-synchronous maturity, seed shattering, yield instability and lack of high yielding cultivars resistant to major insect pests and diseases. To improve the productivity levels of this crop for ensuring the demand of ever increasing population, improvement of yield over existing varieties is necessary by developing high yielding varieties.

Genetic diversity is a pre-requisite for genetic improvement of any crop. Besides the availability of genetic diversity, their characterization is crucial for effective utilization in the crop improvement. In India, though more than 90 varieties were released and notified, still identification of these varieties is challenging. Under the PPV&FR act 2001 of India, characterisation of varieties based on DUS descriptors is mandate for registration of varieties. DUS test is a way for defining whether a newly bred variety diverges from existing varieties with in the species (the distinctive part), whether these distinctive characters are expressed uniformly (the uniformity part) and if these characters do not change over succeeding generations (the stability part). (Anandhi *et al.,* 2015).

The favorable outcome of any crop improvement program basically depends upon the nature and magnitude of genetic variability present in crop. Selection of the genotypes with high variability is critical for creating genotypes with high yield and other desirable features. The efficacy of selection is known by the proportion of heritable variability. In the prediction of projected genetic gain for the trait with selection of best individual genotypes from the base population, the information on heritability combined with genetic advance for yield contributing characters would be of great value.

The knowledge on genetic divergence helps to identify genetically diverse genotypes for any breeding programme. The Mahalanobis D^2 statistics has been found to be a powerful tool in quantifying the degree of genetic

divergence among the genotypes (Mahalanobis, 1936). The use of genetically divergent parents in breeding programme is expected to throw superior and desirable segregants following the crossing programme (Bhatt, 1973). In addition to aiding in the selection of divergent parents, it measures the degree of divergence at genotypic level and determines the relative contribution of each component character to the total divergence.

Characterization of genotypes based on DUS descriptors alone may not be helpful all the times in distinguishing the varieties with common ancestry. Advances in molecular technology have resulted in a trend towards employing genetic markers for discovering individual differences. Molecular profiling is also an effective method for varietal identification. DNA banding pattern developed by using different primers for each genotype serves as its genetic identity, which is more precise, rapid and distinct. Various DNA fingerprinting techniques *viz.,* random amplified polymorphic DNA (RAPD) (Bhat *et al.,* 1999, Salazar *et al.,* 2006), amplified fragment length polymorphism (AFLP) (Laurentin *et al.,* 2007) and simple sequence repeat (SSR) (Thomson *et al.,* 2007, Bhat *et al.,* 2014) can be employed for cultivar identification as per the UPOV guidelines. Among them, SSRs are considered an ideal marker choice for assessing the genetic diversity and germplasm characterization considering their high-fidelity, co-dominant nature, chromosome specificity, high polymorphism and reproducibility (Nandakumar *et al.,* 2004, Kaur *et al.,* 2018).

With this background, the present investigation entitled, "**DUS characterization and molecular diversity analysis in sesame (*Sesamum indicum* L.)**" was undertaken with the following objectives

Objectives of investigation

1. To assess genetic variability and genetic parameters for yield and yield attributing traits
2. To study genetic diversity using both DUS traits and molecular markers
3. To characterize sesame varieties based on DUS traits
4. To develop varietal specific DNA fingerprints using molecular markers

Chapter - II

Review of Literature

Chapter II

REVIEW OF LITERATURE

A brief review of available literature on **"DUS characterization and molecular diversity analysis in sesame" (S*esamum indicum* L.)"** in consonance with the objectives of the present investigation are reviewed and presented under the following heads.

2.1 DUS CHARACTERIZATION

2.2 GENETIC VARIABILITY

2.3 GENETIC DIVERSITY

2.4 MOLECULAR DIVERSITY

2.1 DUS CHARACTERIZATION

The availability of genetic diversity is a pre-requisite for genetic improvement of any crop. Besides the availability of genetic diversity, their characterization is crucial for effective utilization in the crop improvement. DUS test is a way for defining whether a newly bred variety diverges from existing varieties with in the species (the distinctive part), whether these distinctive characters are expressed uniformly (the uniformity part) and these characters do not change over succeeding generations (the stability part). DUS characterization was done according to guidelines provided by the Protection of Plant Variety and Farmer's Right Authority. There are 20 DUS descriptors in sesame as per the national guidelines for the conduct of Distinctness, Uniformity and Stability.

Mahesh (2014) studied 20 sesame genotypes for various plant morphological characteristics and revealed that most of the genotypes have dark green leaves (12 genotypes), light pink flower petal colour (11 genotypes), brown seed colour (7 genotypes), white seed colour (7 genotypes), tall plant height (10 genotypes), medium number of pods (9 genotypes), long pod length (7 genotypes) and many number of leaves (9 genotypes).

Falusi *et al.* (2015) evaluated morphological characteristics for 12 distinct sesame genotypes from different parts of northern Nigeria such as plant height, petiole length, number of leaves per plant, number of branches per plant and leaf surface area per plant and they reported that NG01 had the tallest plants whereas KG01 and NA01 were shortest.

One twenty nine sesame accessions and eight cultivars were assessed by Frary *et al*. (2015) for eight qualitative traits. They observed no variation for plant growth type, stem branching, and capsule dehiscence at ripening and also reported that all plants were indeterminate, branched and completely shattering. Limited variation was observed for hairiness. More than 93% of the accessions had sparse stem, leaf, and capsule hairiness. Only one cultivar, Orhangazi 99, had moderate stem and leaf hairiness with profuse capsule hairiness.

Azeez *et al.* (2016) evaluated 17 sesamum genotypes for morphological characters and revealed that most of the floral characters are with narrow diversity and moderate variability observed in characters like flower length, style length, capsule length and number of seeds per capsule.

Ukani (2018) characterized 25 sesame genotypes based on the morphological characters and molecular markers. Most of the genotypes exhibited medium days to 50 % flowering (19 genotypes), light purple flower petal colour (25 genotypes),sparse flower petal hairiness (13 genotypes), medium plant height (23 genotypes) , few number of primary branches per plant (18 genotypes), basal branching (25 genotypes), absence of stem hairiness (25 genotypes), weak leaf serration of margin (23 genotypes) ,sparse capsule hairiness (15 genotypes) , broad oblong capsule shape (20 genotypes) , single capsule per leaf axil (16 genotypes) , opposite capsule arrangement (13 genotypes), moderate number of capsules per plant (15 genotypes), long capsule length (17 genotypes), late days to maturity (19 genotypes) and white seed colour (23 genotypes).

Bhoot *et al*. (2019) evaluated 30 genotypes of sesame for various plant and seed morphological traits and noticed that the most of the genotypes have

basal branching (23 genotypes), late maturity (20 genotypes), absence of stem hairiness (26 genotypes), more nodes (27 genotypes), slightly lobed leaves (30 genotypes) and more leaves (22 genotypes).

Mawcha *et al*. (2020) studied ten nationally released sesame genotypes based on agro morphological traits and revealed that number of primary branches per plant was higher for M-80. The number of nodes per plant in all the genotypes was grouped as more nodes per plant *i.e.,* greater than 15. The variation was least in qualitative characteristics like flower colour, number of locules per pod, pod pubescence and type of pod beak. Pod length was found to be varied among the genotypes and grouped as long with a mean pod length of 2.44 cm.

Palakshappa *et al.* (2020) characterized seven hundred and twenty accessions of sesame for twenty morphological descriptors. Most of the genotypes showed determinate growth type (63.1 %), semi erect growth habit (42.7 %), sparse stem hairiness (52.3 %), basal branching (51.6 %), weak leaf hairiness (48.3%), linear leaf shape (64.0 %), absence of lobe incision in leaf (47.0 %), green petiole colour (99.2 %), one flower per axil (86.7 %), sparse corolla hairiness (38.9 %), four locules per capsule (95.7 %), narrow oblong capsule shape (47.6 %), monocapsular arrangement (94.4 %), glaborous capsule hairiness (34.6 %), brown colour of dry capsule (49.3 %), long capsule break (29.0 %) and beige seed coat colour (26.3 %).

Savaliya (2021) studied forty black sesame genotypes for 26 plant morphological characters. Most of the genotypes had medium days to 50 per cent flowering (35 genotypes), light purple flower petal colour (32 genotypes), sparse flower petal hairiness (26 genotypes), medium plant height (23 genotypes), medium number of primary branches per plant (19 genotypes), basal branching pattern (33 genotypes), very late days to maturity (31 genotypes), absence of hairiness of stem (34 genotypes), slightly lobes of leaf (40 genotypes), medium length of leaf (21 genotypes), weak serration margin of leaf (34 genotypes), sparse capsule hairiness (30 genotypes), four locules per

capsule in (37 genotypes), broad oblong capsule shape (18 genotypes), single capsule per leaf axil(36 genotypes), opposite capsule arrangement (25 genotypes), moderate number of capsules per plant, (33 genotypes) and medium capsule length (30 genotypes) .

Seventy soybean genotypes were evaluated by Sivabharathi *et al*. (2022) based on DUS guidelines and recorded that most of the genotypes have presence of hypocotyl anthocyanin pigmentation (80 %), semi determinate plant growth (34 %), pointed ovate leaf shape (77 %), green leaf colour (91 %), erect growth habit (72 %), purple flower colour (80 %), tawny pod pubescence colour (80 %), spherical seed shape (58 %) and yellow seed colour (98 %).

Barela *et al*. (2022) characterized ninety soybean genotypes for sixteen DUS traits and revealed that most of the genotypes have frequency distribution of hypocotyl anthocyanin pigmentation (65.55 %), semi-determinate growth type (75.55 %), semi-erect growth habitat (90.0 %), pointed ovate leaf shape (83.33 %), pod hairiness pubescent (84.44%), medium seed size (64.44%), yellow seed coat colour (84.44%) and brown colour of funicle (100%).

Mathur *et al*. (2022) studied morphological characteristics of 16 sesamum varieties from different regions of India for DUS testing. Among all the varieties, TMV-5 showed few (1-2) branches and large leaf size unlike other 15 varieties which had medium to many branches and small to medium leaf size. GT-1 showed cluster capsule arrangement while all fifteen showed alternate or opposite capsule arrangement and Adarsh-8 showed six locule number inside the capsule while other fifteen varieties studied showed four locule number.

Vanishree *et al*. (2022) characterized 457 germplasm accessions of sesame based on the DUS morphological descriptors. On the basis of frequency distribution, majority of sesame accessions were found to possess determinate plant growth type (56.9%), erect plant growth habit (49.5%), sparse stem hairiness (61.9%), basal branching pattern (53.6%), weak leaf hairiness (42.2%), entire basal leaf margin (43.3%), no lobe incision of basal leaf

(38.9%), one flower per leaf axil (58.6%), sparse corolla hairiness (45.3%), white exterior corolla colour (40.0%) , white interior corolla colour (43.5%), four locules per capsule (96.9%), narrow oblong bicarpellate capsule shape (44.0%), monocapsular capsule arrangement (90.6%), weak capsule hairiness (53.4%),long capsule beak (30.9%) and beige seed coat colour (22.1%).

2.2 GENETIC VARIABILITY

Genetic improvement of any crop largely depends upon the magnitude of genetic variability, since it offers scope for natural and artificial selection to tailor suitable genotypes for diverse agroclimatic conditions. Variability results due to differences either in the genetic constitution of the individuals of a population or environment in which they are grown. The coefficient of variation expressed at phenotypic (PCV) and genotypic (GCV) levels are used to compare the variability observed among the different characters. Estimation of genetic variability does not give a clear indication of the possible improvement and can be attained through selection in connection with heritability and genetic advance. The available literature on variability studies in sesame is summarized below.

Menzir *et al*. (2012) studied 64 sesame genotypes for variability parameters. They reported that number of branches per plant, number of capsules per plant and seed yield per hectare exhibited high phenotypic coefficient of variation while high heritability was recorded by days to maturity followed by 1000 seed weight and oil content while the traits number of branches per plant and plant height noted moderate heritability coupled with high genetic advance as per cent of mean

In a genetic variability study involving 81 sesame genotypes for 15 characters by Gidey *et al.* (2013) deciphered higher estimates of genotypic and phenotypic Coefficient of Variation for harvest index, seed yield per hectare, height to first capsule, biomass per hectare, number of capsules per hectare, number of primary branches per hectare, number of seeds per capsule and plant height. Height to first capsule had the highest heritability value. High

heritability coupled with high genetic advance as percent of mean was observed for number of primary branches per plant, height to first capsule and harvest index.

Bharathi *et al*. (2014) investigated genetic variability among 50 sesame accessions and revealed that genotypic coefficient of variation and phenotypic coefficient of variation was moderate to high for seed yield per plant, number of capsules per plant and number of branches per plant. The traits *viz*., days to maturity, days to 50 % flowering, seed yield per plant, number of capsules per plant and number of branches per plant recorded high heritability. They also noticed high heritability coupled with high genetic advance for seed yield per plant, number of capsules per plant and number of branches per plant

Bindu *et al*. (2014) studied 31 sesame genotypes and reported that high level of genotypic coefficient of variation and phenotypic coefficient of variation in number of branches per plant, number of capsules per plant and seed yield per plant. High heritability and high genetic advance as per cent of mean was recorded for almost all the traits *viz*., plant height, number of branches, number of capsules per plant, oil content and yield except days to 50 % flowering and 1000 seed weight.

Hika *et al*. (2015) determined the extent of genetic variability among sixty four sesame populations and noted that the traits *viz*., days to 50% flowering, plant height, number of branches per plant, number of capsules per plant, 1000 seed weight, seed yield and harvest index showed highly significant differences among the populations. High genotypic coefficient of variation and phenotypic coefficient of variation was observed for number of primary branches per plant, number of branches per plant, seed yield and harvest index. High to very high estimates of heritability values were observed for all characters except for 1000-seed weight.

Twelve genotypes of sesame were evaluated for genetic variability by Sabiel *et al*. (2015) and observed that high genotypic coefficient of variation for seed yield and days to flowering showed high heritability estimate.

Moreover, the high genetic advance was recorded in 1000-seed weight while all other traits showed low genetic advance.

In a genetic variability study of sixty sesame genotypes by Soundharya *et al*. (2016) found that highly significant differences among the genotypes for all traits. Number of branches per plant exhibited the highest genotypic coefficient of variation and phenotypic coefficient of variation followed by number of capsules per plant and harvest index. High heritability coupled with high genetic advance as per cent mean was observed for plant height, number of branches per plant, number of capsules per plant, number of seeds per capsule, 1000 seed weight, seed yield per plant and harvest index.

Tripathy *et al*. (2016) estimated variability in twelve genotypes of sesame and they reported high genotypic coefficient of variation for number of primary branches per plant followed by seed yield per plant, number of capsules per plant, number of seeds per capsule and height to first capsule. Number of seeds per capsules exhibited high heritability coupled with high genetic advance. Similarly, number of primary branches per plant, height to first capsule and seed yield exhibited moderate heritability coupled with high genetic advance. In contrast, low heritability as well as low genetic advance was observed for 500-seed weight.

Abhijatha *et al*. (2017) studied genetic parameters in 33 genotypes of sesame for 12 morphological characters. High to moderate GCV and PCV were noticed for most of the yield contributing characteristics except for 1000-seed weight. Higher estimates of heritability coupled with high to moderate genetic advance as per cent over mean was recorded by all the yield associated characters except for days to maturity, oil content and 1000 seed weight.

Saxena and Bisen (2017) evaluated variability in 14 advanced breeding lines of sesame. High values for genotypic coefficient of variation and phenotypic coefficient of variation was recorded for seed yield per plant followed by number of primary branches per plant, oil content and seeds per capsule. High heritability was recorded for seeds per capsule followed by oil

content (%), yield per plant (g) and harvest index (%). High heritability combined with high genetic advance was recorded for seeds per capsule followed by oil content (%), yield per plant (g) and harvest index (%).

Kiruthika *et al*. (2018) assessed 53 sesame genotypes for 18 morphological characters and noticed that GCV was higher than PCV for most of the characters. The maximum genotypic coefficient of variation was observed for number of secondary branches whereas it was minimum for capsule width. All the characters invariably showed high heritability except capsule width and single plant yield, while plant height exhibited high heritability with moderate genetic advance as per cent of mean.

In a genetic variability study of 100 sesame advanced breeding lines for 11 quantitative characters, higher estimates of phenotypic coefficient of variation were observed for all the characteristics. High heritability coupled with high genetic advance was observed for plant height and distance from ground to first capsule. (Patil and Lokesha 2018).

The investigation carried out by Singh *et al*. (2018) in 75 sesame genotypes reported that high value for genotypic coefficient of variation and phenotypic coefficient of variation for number of secondary branches per plant followed by number of primary branches per plant, seed yield per plant, number of seeds per capsule and number of capsules per plant. High heritability coupled with moderate genetic advance was recorded for oil content and days to 50 % flowering.

Kalaiyarasi *et al*. (2019) investigated genetic parameters of 13 genotypes of sesame for eight quantitative characters and reported high GCV and PCV for seed yield per plant, number of capsules per plant and number of branches per plant. Moreover, high heritability coupled with high genetic advance was observed for the characters *viz*., seed yield per plant, number of seeds per capsule, number of capsules per plant, number of primary branches per plant and plant height.

Sultana *et al*. (2019) evaluated fifty diverse sesame genotypes and revealed that, the phenotypic variance and phenotypic coefficient of variation higher than the genotypic variance and genotypic coefficient of variation for all the traits. Higher GCV and PCV were recoded for seed yield per plant, pods per plant, plant height and seeds per pod. High heritability was found in 100-seed weight followed by days to 80 % maturity, pods per plant and seed yield per plant.

Kadvani *et al*. (2020) studied genetic variability, heritability and genetic advance for 11 characters in 45 genotypes of sesame. Phenotypic coefficient of variation were slightly higher than that of genotypic coefficient of variation for all the traits. High genotypic and phenotypic coefficient of variation were recorded for seed yield per plant, leaf area per plant and number of effective branches per plant. High heritability was noted for 1000 seed weight, leaf area per plant, oil content, days to flowering and capsule length. High heritability combined with high genetic advance as percentage of mean was recorded for leaf area per plant, seed yield per plant, number of effective branches per plant and number of capsules per plant.

Kehie *et al*. (2020) studied 25 genotypes of sesame for twelve quantitative characters. The analysis of variance indicated the existence of significant variations among the genotypes for all the characters except for plant height, stem height from base to first branch and number of locules per capsule. The genotypic coefficients of variation for all the characters were lesser than the phenotypic coefficient of variation. High heritability coupled with high genetic advance as per cent mean was observed for number of capsules per plant, seeds per capsule, 1000 seed weight, days to 50 % flowering and oil content.

Twenty four sesame genotypes assessed variability for 10 agro morphological characters, analysis of variance revealed considerable variability among all the characters. The phenotypic coefficient of variation was greater than genotypic coefficient of variation for all the characters. High heritability

coupled with high genetic advance were observed for number of branches per plant, biological yield per plant, harvest index and seed yield per plant. (Manjeet *et al*., 2020).

Kant *et al*. (2021) evaluated ten genotypes for ten morphological traits and revealed that number of capsules per plant recorded high GCV and PCV. High heritability was observed for the characters *viz.,* number of seeds per capsule, days to 80 % maturity and number of primary branches per plant. High heritability coupled with high genetic advance was recorded for seed yield per hectare, days to 80% maturity and number of capsules per plant. High heritability coupled with low genetic advance was recorded for capsule length, oil content and 1000-seed weight.

Vamshi *et al*. (2021) estimated genetic parameters in twenty seven sesame breeding lines along with three checks for twelve different traits, analysis of variance (ANOVA) indicated significant variation among the genotypes for all the characters except for capsule width. The GCV for all the characters were lesser than the PCV. High heritability coupled with high genetic advance as percent of mean was observed for three characters *viz.,* oil content, plant height and seed yield per plot. High heritability estimates coupled with moderate genetic advance were manifested in three other traits *viz.,* days to 50 % flowering, test weight and seed weight per capsule.

In a genetic variability study of 33 sesame genotypes including three checks for 11 traits. The magnitude of genotypic coefficient of variation and phenotypic coefficient of variation was high for seed yield, number of productive branches and inter-node length. The traits *viz.,* seed yield, number of productive branches and height of first node from ground and internode length exhibited high genetic advance as per cent of mean.(Kumar *et al*., 2022)

Twenty nine sesame genotypes were evaluated for genetic variation by Roy *et al*. (2022) based on morphological traits. The high genotypic coefficient of variation and phenotypic coefficient variation observed for number of branches per plant and number of capsules per plant. High heritability coupled

with high genetic advance was noted against seed yield per plant, number of branches per plant, capsules per plant and harvest index.

Saha *et al*. (2023) analysed 29 sesame genotypes for 11 morphological traits for genetic variability. GCV was found to be highest for seed yield per plant followed by number of branches per plant and number of capsules per plant. Moderate GCV and PCV were observed for capsule length, plant height, number of seeds per capsule and days to 50 % flowering, whereas low GCV and PCV were observed for days to maturity. High broad sense heritability coupled with high genetic advance was reported for number of capsules per plant followed by number of branches per plant.

Takele *et al*. (2023) studied twentyfive sesame genotypes for 12 quantitative characters and revealed that there was significant differences among the genotypes for all traits except for days to maturity and biomass yield per hectare. High genotypic and phenotypic coefficient of variation, heritability and genetic advance as percent mean was exhibited by biomass yield per plant and harvest index while capsule per plant, seed yield per plant and seed yield per hectare showed moderate phenotypic and genotypic coefficient of variation and high heritability with genetic advance as percent mean. Branches per plant showed medium phenotypic and genotypic coefficient of variation, heritability and genetic advance as percent mean. Days to flowering, plant height and capsule length showed low phenotypic and genotypic coefficient of variation and genetic advance as percent mean.

2.3 GENETIC DIVERSITY

Genetic divergence is a prerequisite for hybridization programme as it serves as a guide in the choice of parents because crosses between divergent parents usually produce greater heterosis than those between closely related ones. Genetic divergence is a result of changes in gene frequencies of different populations due to evolutionary forces. The concept of D^2 statistics for measuring the divergence between the two populations was introduced by Mahalanobis (1936). Use of diverse parents in hybridization programme can serve the purpose of combining desirable genes or to obtain recombination.

Parameshwarappa *et al*. (2012) studied the genetic divergence in 131 germplasm accessions of sesame adopting Mahalanobis's D^2 statistic and reported that seed yield, plant height and seeds per capsule are the major contributors to the genetic divergence. Grouping of genotypes into clusters using Tocher's method resulted in formation of eight clusters. Maximum intracluster distance was shown by cluster IV while, cluster II and VI showed highest inter cluster distance. Cluster VII exhibited highest means for seed yield and number of capsules per plant.

Narayanan and Murugan (2013) studied sixteen sesame genotypes for genetic divergence using Mahalanobis D^2 statistics and the genotypes were grouped into 8 clusters. Maximum inter cluster distance was observed between the clusters III and VII. Seed yield contributed the maximum towards the divergence followed by number of pods per plant, days to 50 % flowering and plant height.

On the basis of D^2 analysis, Bisen *et al*. (2013) grouped the sesame germplasm lines into eleven clusters. Highest inter cluster distance was observed between cluster VI and cluster XI followed by clusters V and XI while, lowest distance was noticed between cluster IV and V. Days to 50 % flowering contributed highest towards genetic divergence followed by seed yield per plant.

Mohan *et al*. (2014) studied on diversity of sesame using Mahalanobis D^2 statistic, the genotypes were grouped into 12 clusters and highest inter cluster distance was observed between cluster VI and XII followed by cluster VI and IX, cluster II and VI and cluster III and IV. Among the traits studied capsule per plant and plant height contributed maximum towards the divergence.

Tripathi *et al*. (2014) using D^2 analysis grouped the sesame genotypes into eleven clusters. Maximum inter cluster distance was observed between cluster VI and cluster XI followed by cluster V and XI, whereas minimum distance was recorded between cluster IV and V. Among all the nine traits, days to 50 % flowering contributed highest towards genetic divergence.

Amit *et al*. (2015) grouped 80 germplasm accessions into eight clusters based on Mahalanobis D^2 statistic. Maximum number of accessions were found in cluster I followed by cluster III, cluster V, cluster II, cluster IV, cluster VII and cluster VIII with one germplasm accession each. Maximum inter cluster distance was found between cluster V and cluster VIII followed by cluster I and cluster VIII. Maximum intracluster distance was observed for cluster VI followed by cluster II and cluster V.

Genetic diversity assessment of 13 sesamum genotypes using Mahalanobis D^2 analysis grouped the genotypes into 4 clusters. Maximum number of genotypes were in cluster I with nine genotypes followed by cluster IV with two genotypes, while remaining clusters II and III were solitary each. Maximum inter cluster distance was found between cluster II and III, whereas, the lowest distance was found between clusters I and III. (Fiseha *et al*., 2015)

Bamrotiya *et al*. (2016) assessed the genetic diversity among 40 genotypes of sesame and classified them into three clusters. Cluster I had 36 genotypes, while cluster II contained three genotypes whereas cluster III possessed only one genotype. Height to first capsule contributed maximum to the genetic divergence followed by number of capsules per leaf axil, length of capsule, seed yield per plant and number of seeds per capsule.

Soundharya *et al*. (2017) applied Mahalanobis's D^2 statistics to access the divergence among the 62 sesame genotypes. All the genotypes were grouped into six clusters where, cluster I was largest containing 44 genotypes followed cluster II with twelve genotypes, cluster VI with three genotypes and cluster III, V and IV had only one genotype. The inter cluster distance was maximum between cluster IV and VI wheras minimum between cluster I and III.

Thirty sesame genotypes were evaluated for genetic diversity by Suganthi *et al*. (2017) for nine yield and yield attributing characters using Mahalanobis D^2 statistics and the genotypes were grouped nine clusters. Inter cluster distance was maximum between VII and VIII followed by cluster I and

VIII, whereas minimum intercluster distance was found between cluster V and VI. Plant height at maturity, number of capsules per plant and number of seeds per capsule contributed maximum towards divergence. The clusters III and I recorded maximum cluster mean value for seed yield per plant.

The diversity study carried out by Patil *et al*. (2018) for 100 sesame advanced breeding lines using Mahalanobis's D^2 analysis grouped lines into 12 clusters. The intra cluster distance was maximum in cluster III though it had only 14 entries but it was lowest in cluster I despite having of 76 entries. Inter cluster distance was maximum between cluster III and XII. The maximum divergence was contributed by height from ground to first capsule followed by days to 50 % flowering, number of capsules per plant and days to maturity.

Swathy *et al*. (2018) evaluated 90 genotypes of sesame using Mahalanobis's D^2 statistics. Among the nine clusters formed, highest inter cluster distance was between cluster IX and V whereas it was minimum between cluster VIII and II. Cluster means for number of capsules per plant and seed yield per plant were highest in cluster IX while cluster III had highest for oil content and days to 50 % flowering.

Tanwar and Bisen (2018) conducted an experiment with 97 diverse genotypes of sesame which clustered all the genotypes into 15 groups based on Mahalanobis D^2 statistics. Cluster I was the largest among all clusters comprising 48 accessions. The highest intra cluster was recorded in cluster V and the inter cluster distance was highest between the cluster VI and cluster XV followed by cluster VIII and cluster XV.

Arpitha *et al*. (2019) selected 270 germplasm lines to study the genetic diversity using Mahalanobis D^2 statistics for six biometrical traits. A total of sixteen clusters were obtained using Tocher's method. Out of sixteen clusters, cluster XIV and XV showed highest inter cluster distance whereas clusters II and V registered lowest distance. The highest cluster mean was observed in cluster XIV for the trait seed yield per plant.

Ramprasad *et al*. (2019) studied genetic diversity for 41 sesame genotypes and grouped them into seven clusters. The maximum diversity was contributed by capsules per plant followed by number of branches per plant, plant height and days to maturity. The inter cluster distance was higher than the intra cluster distance. Four characters *viz*., capsules per plant followed by number of branches, plant height and days to maturity contributed more than 90 % towards genetic divergence.

Kabi *et al*. (2020) assesed genetic diversity in thirty sesame genotypes for diversity analysis for thirteen quantitative traits. The thirty genotypes were grouped into nine clusters based on the Mahalanobis D^2 values following Tocher's method. Maximum inter cluster distance was exhibited between cluster IV and cluster VI and intra cluster distance was in cluster III.

Genetic diversity among thirty sesame genotypes using Mahalanobis D^2 statistics revealed that all genotypes were grouped into four clusters of which Cluster I had 21 genotypes, cluster II, cluster III and cluster IV were monogenotypic. Maximum inter cluster distance was recorded between cluster III and IV. (Mohanty *et al.*, 2020)

Tesfaye *et al*. (2021) identified the genetic diversity in three hundred cultivated sesame genotypes using Mahalanobis D^2 statistics, and the genotypes were grouped into six different clusters. Maximum inter cluster distance was observed between clusters IV and VI, followed by clusters I and VI.

Srikanth *et al*. (2022) studied genetic divergence against the 65 diverse germplasm of sesame using Mahalanobis D^2 statistics, all the genotypes were clustered in 4 groups. Cluster I was the largest among all clusters comprising 60 germplasm. The trait 1000-seed weight contributed maximum towards diversity followed by days to maturity. The highest intra cluster distance was found in cluster II followed by cluster I. The maximum intercluster distance was found between cluster I and IV while minimum inter-cluster distance was observed between clusters I and III.

In a genetic diversity study of 64 sesame germplasm based on the Mahalanobis D^2 statistics for eleven characters noticed that maximum intra cluster distance was observed in cluster I followed by cluster XII. Cluster XVI exhibited high mean value for number of branches per plant, 1000-seed weight, oil content and seed yield per plant. Oil content showed maximum contribution followed by number of capsules per plant, plant height, seed yield per plant, number of seed per capsule and 1000-seed weight (Uikey *et al*., 2022)

Yadav *et al*. (2022) used Mahalanobis D^2 analysis to study the genetic diversity of 50 sesame genotypes. The genotypes were grouped into nine clusters based on ten economically important traits.The largest intercluster distance was found between clusters I and VIII. The highest cluster mean was found in cluster VIII for the number of capsules per plant, the number of primary and secondary branches, the number of seeds per capsule and single plant yield and cluster V for plant height and cluster VII for thousand seed weight.

Sasipriya *et al*. (2023) assessed genetic diversity among the 51 sesame genotypes using Mahalanobis's D^2 statistics. All the genotypes were grouped into ten clusters and out of them, cluster I was the largest comprising of eighteen genotypes followed by cluster III with fifteen genotypes, cluster IV with seven genotypes, cluster II with five genotypes while the clusters *viz*., V, VI, VII, VIII, IX and X were` represented by single genotype. The maximum inter cluster distance was observed between cluster IX and cluster X followed by cluster VII and cluster VIII. Days to 50% flowering contributed maximum towards divergence followed by seed yield per plant, capsule length and number of seeds per capsule.

Sixty eight sesame genotypes, including three checks subjected to genetic diversity through D^2 statistic and distributed into eight clusters. Among the eight clusters, cluster I was the largest comprising of 30 genotypes followed by cluster II with 22 genotypes and Cluster III and V with six genotypes in each and remaining clusters IV, VI, VII, VIII were solitary. The maximum

contribution towards genetic divergence was by days to 50 % flowering, number of capsules per plant, number of seeds per capsule, number of branches per plant and seed yield per plant. (Swapna *et al.,* 2023).

2.4 MOLECULAR DIVERSITY

The variety identification serves the important goals such as mitigating legal claims, confirming Intellectual Property Rights and maintenance of genetic purity. The current plant variety identification for their protection relies on morphological description of plant varieties. However, establishing identity of varieties based on the DUS descriptors alone may not be reliable due to their limited number, stage specificity and environmental influence which complicate their evaluation. In contrast, DNA or molecular markers which are free from the environmental effects can be applied at different stages of plant growth (Noli *et al.,* 2008).

The increased availability and cost-efficiency of DNA-based markers in recent years makes them an attractive option to explore their use and to supplement or even ultimately replace the existing morphological and protein-based approaches (Jamali *et al*., 2019). Among the DNA based markers, microsatellite or Simple Sequence Repeat (SSR) markers were mostly used by many of the scientists to identify the genotypes. DNA fingerprinting or profiling developed by using molecular markers to describe the combined use of several single locus detection systems is being used as a versatile tool for characterization of sesame genotypes. Owing to these reasons, the International Union for the Protection of New Varieties of Plants (UPOV), an inter governmental organization whose system of plant variety protection is intended to encourage innovation in the field of plant breeding (Jordens, 2005) has called for the adoption of a DNA-based system that will enable examiners to deploy trait-specific DNA markers in DUS testing (Jamali *et al*., 2019)

Kola *et al*. (2012) determined the level of genetic diversity among nine sesame genotypes using 207 sesame-specific microsatellite markers. Of these, 46 markers were polymorphic. A dendrogram from the molecular profiles of 46

polymorphic primers showed four clusters and one clade. Among these, the clade included ‘Swetha’, cluster I included ‘Rajeswari’, ‘Madhavi’, cluster II included ‘Chandana’, ‘YLM-11’, and ‘Hima’, cluster III included ‘YLM-17’, and cluster IV included ‘YLM-66’ and ‘Gouri’. The genetic similarity coefficient among the 9 genotypes ranged from 79 to 92 % and PIC values ranged from 0.41 to 0.96. By DNA fingerprinting, all 9 genotypes could be easily distinguished with only six SSR primers.

Zhang *et al*. (2012) studied the characterization of 12 sesame germplasm accessions from China using 10 SSR markers and reported that three SSR markers showed polymorphism. A total of 175 bands were obtained. 126 out of 175 bands were polymorphic. The number of alleles ranged from 2 to 17 with an average of 9. Similarity coefficient values were ranged between 0.43 and 0.98 with an average of 0.7.Shannon’s diversity index ranged between 0.3 and 0.4.

Islam *et al*. (2013) used four varieties of sesame for fingerprinting by amplified polymorphic DNA (RAPD) markers. A total of 21 PCR amplification products were found by using three decamer primers (OPA-09, OPC-OS and OPL-07) among which 14 bands were polymorphic. The size of the amplification products ranged from 347bp to 1224bp for OPA-09, 317bp to IS92bp for OPC-OS and 423bp to 1024bp for OPL- 07 primer. No genetic variation was observed within the individuals of single variety.

Yepuri *et al*. (2013) used 156 SSR markers to determine the diversity among 49 sesame accessions. Only 20 SSRs showed polymorphism and the number of alleles per primer ranged from two to five. The allele size varied from 101 to 399 bp and the average PIC values of the 20 SSR loci was 0.72 with the range of 0.49 to 0.90. The genetic similarity coefficient values ranged from 0.59 to 1.0.

Sixteen sesame genotypes were assessed using 120 SSR markers and the results indicated that 92 markers were polymorphic. The number of alleles per microsatellite locus ranged from 2 to 5 with an average of 3.11 alleles. The

allele size ranged from 100–510 bp. PIC estimates ranged from 0.298 to 0.912 and Jaccard's similarity coefficient values ranged from 0.21 to 0.82. (Badri *et al.,* 2014).

Surapaneni *et al*. (2014) tested 68 sesame accessions and three wild species using 102 SSR primers. Among these markers, 72 SSR markers exhibited polymorphism. The results detected 170 alleles with an average of 2.5 alleles per locus and the number of alleles ranged from 2 to 4. PIC values of the markers ranged from 0.43 to 0.88 with an averageof 0.66 and genetic similarity coefficient values were ranged from 0.40 to 0.91.

Abate and Mekbib (2015) screened 128 sesame genotypes using seven ISSR primers and revealed that the primers yielded 89 polymorphic reproducible bands out of 96 total amplified bands.The number of amplified bands varied from 7 to 19 with an average number of bands 14 and the polymorphic bands per primer were 12.6. The PIC values ranged between 0.26 and 0.76 showing the high informativeness of the selected primers.

Forty sesame accessions were analysed by Singh *et al*. (2015) with 12 SSR markers to know the patterns of genetic variation among the accessions. The range of polymorphism information content values was 0.43 to 0.74 with an average value of 0.55. The 12 selected SSR primers generated 41 amplified bands with the fragments size varied from 155 to 371 bp. Expected heterozygosity of SSR primers ranged from 0.404 to 0.740.

Kiranmayi *et al*. (2016) estimated molecular diversity in 23 sesame accessions using 10 SSR markers. Molecular analysis revealed 4 polymorphic primers. A total of 14 alleles were detected at four loci with an average of 3.5 alleles per locus. PIC values ranged from 0.28 to 0.78 with an average of 0.53. The pair wise similarity coefficient values ranged from 0.2 to 0.7 with an average of 0.45.

Forty seven sesame accessions were subjected to 18 SSR primers and all the primers showed polymorphism and gave 64 polymorphic DNA bands with band size of 150 to 1500bp. The total number of alleles ranged from 1 to 9

with an average of 3.55. PIC value ranged between 0.09 and 0.36 with an average of 0.19. The Jaccard's similarity coefficient values ranged from 0.16 to 0.86 with an average of 1.03. (Dar *et al*., 2017).

Sixty nine commercially cultivated and released varieties of sesame were studied by Kumari *et al*. (2017) for molecular characterization and diversity using 12 SSR and 10 ISSR markers. Out of 12, six SSR markers showed polymorphism. The number of alleles per locus produced by SSR markers ranged from 1 to 6 with an average of 4.83. Average pair wise genetic similarity ranged from 0 to 0.625.

Ramprasad *et al*. (2017) examined 41 sesame genotypes with a set of 75 sesame SSR primer pairs to assess the molecular diversity. Out of 75 SSR primer pairs, 20 were polymorphic (29.4 per cent polymorphism) primers. The 41 sesame genotypes were broadly divided into three clusters based on dendogram constructed by weighted Neighbor-Joining method

Bhattacharjee *et al*. (2018) analysed the variety CUMS 17 (Suprava) along with two check varieties namely GT-10 and Savitri by using 21 SSR primers, among them 14 polymorphic SSR primers were used for DNA fingerprinting. The SSR primers produced a total of 46 alleles with an average number of 3.28 alleles with amplicon size ranging from 100 to 600 bp.

Gogoi *et al*. (2018) evaluated 33 indigenous sesame genotypes for genetic diversity using 50 SSR markers, of which 49 were polymorphic (62.82%) with an average of 2.89 alleles per locus ranged from 1 to 5. The PIC values varied from 0.01 to 0.99 witha mean of 0.43. The similarity coefficient values ranged from 0.931 to 0.591 with an average of 0.754.

Genetic diversity in sesame was carried out using NTsys analysis and clustered the 30 genotypes into five main clusters based on 21 polymorphic SSR primers which revealed the presence of 93 alleles. The number of alleles per microsatellite locus varied from 3 to 6 with an average of 4.42 alleles. The size of amplified product was 80.69 bpto 305.8 bp. PIC value estimated ranged from 0.07 to 0.87. (Bhattacharjee *et al*., 2019)

Five SSR primers were used for fingerprinting of the eleven sesame genotypes generated 19 bands, 10 of them were polymorphic with 53% polymorphism. Primer SSR1 produced five bands, four of them were polymorphic. Primer SSR2 and SSR3 generated two bands with 0% polymorphism. Primer SSR 4 revealed three bands one of them was polymorphic. Primer SSR5 showed seven bands, five of them were polymorphic. The highest level of polymorphism was observed in primer SSR1 which showed 80 % polymorphism, while the lowest polymorphism was 0 % in primer SSR2 and SSR3. (Mourad *et al*., 2019)

Pavani *et al*. (2020) characterized 30 sesame genotypes with 50 SSR primers for characterization. Out of which, 45 primers showed clear banding pattern. Among them, only seven primer pairs showed polymorphism and total numbers of bands produced were 191. The number of alleles per locus was varied from 2 to 6 with an average of 3.57. The size of scoring bands ranged from 120-300 bp. PIC value of primer pairs ranged from 0.28 to 0.80 with an average of 0.47. The number of effective alleles ranged from 1.38 to 4.86 with an average of 2.49. The dendogram analysis grouped 30 genotypes into four clusters and one clade exhibiting genetic similarity coefficient values of 0 to 1.0.

Sasipriya *et al*. (2020) studied 45 sesame genotypes with 50 SSR markers by NTsys analysis and the genotypes were grouped into six main clusters using 30 polymorphic markers. The polymorphism information content (PIC) value of SSR loci ranged from 0.5209 to 0.9128 with mean of 0.3940.

Bal *et al*. (2021) evaluated fifty sesame accessions with 10 simple sequence repeat (SSR) markers to assess the extent of genetic diversity and formed two clusters. Thirty five alleles revealed mean polymorphism information content of 0.42. These primer bands size varied from 200 to 400 bp. The number of alleles per locus in selected accessions varied from 3 to 6 and heterozygosity per primer ranged from 0.00 to 0.40. The pair wise genetic similarity varied from 0.44 to 0.86.

Mukhthambica *et al*. (2023) characterized 70 genotypes of sesame for 12 morphological as per the DUS guidelines and 14 quantitative characters and subjected to genetic divergence (UPGMA hierarchial clustering) analysis. 70 genotypes were grouped into seven clusters at the genetic distance of 0.5 using Ward's minimum variance method and Gower's method of genetic distance. They also noticed that considerable amount of genetic variability for majority of the morphological traits except for locule number per capsule, capsule number per leaf axil and capsule shape.

Chapter - III

Material and Methods

Chapter III

MATERIAL AND METHODS

The experimental material used and methods followed pertaining to the present investigation entitled **"DUS characterization and molecular diversity analysis in sesame (*Sesamum indicum* L.)"** were briefly described here under.

3.1 LOCATION OF THE EXPERIMENTAL SITE

The experiment was carried out during *Rabi,* 2022-23 at Regional Agricultural Research Station, Tirupati located at 13.62°N latitude and 79.37°E longitude from an altitude of 182.9 m above mean sea level, and situated in southern agro-climatic zone of Andhra Pradesh and molecular characterization was carried out in the Department of Genetics and Plant Breeding, S.V Agricultural college, Tirupati.

3.2 MATERIAL

The experimental material utilized for the present study comprised of 43 released sesame genotypes from different states of India. The lists of genotypes were furnished in the Table 3.1.

3.3 METHODS

3.3.1 Field Layout

The experiment was laid out in a Randomized Block Design with three replications. Each genotype was sown in three rows of three meters length with a spacing of 40 cm between rows and 15 cm between the plants.

Table 3.1 List of 43 genotypes of sesame

S.No	Genotype	Pedigree	Source
1	Pratap	Selection from local collection of Kota district	ARS, Bikaner
2	TKG-21	Punjab Til-1 × TC-25	ZARS, Tikamgarh
3	RT-54	A 6-5 × BS 6-1	RRS, Mandor
4	RT-46	T-12 × Punjab Til-1	RRS, Mandor
5	JCS-2454	Swetha til x Western	RARS, Jagtial
6	CUMS-17	Mutant of IC-21706	Institute of Agricultural science, Kolkata
7	JTS-8	OMT-10 × TC-289	ZARS, Tikamgarh
8	RT-351	NIC-8409 × RT-127	RRS, Mandor
9	Chandana	T-85 × L-5107	RARS, Jagtial
10	VRI-2	VS-9003 × TMV-6	RRS,Vridhachalam
11	RT-125	Type-13 × RT-1	RRS, Mandor
12	RT-346	RT-127 × HT-24	RRS, Mandor
13	RT-390	RT-346 × Es-40 A	RRS, Mandor
14	G.Til-2	G.Til -1 × TC-25	ARS, Amreli
15	Hima	No.5039 × AT-1	RARS, Jagtial
16	RT-127	SI-3500 × Patan-64	RRS, Mandor
17	Gouri	Selection from local germplasm	RARS, Jagtial
18	YLM-11	Vinayak × Kanak	ARS, Yellamanchili
19	YLM-17	Vinayak × Kanak	ARS, Yellamanchili
20	G.Til-1	Selection from MT-67-52	ARS, Amreli
21	G.Til-3	G.Til -1 × AHT-85	ARS, Amreli
22	YLM-66	YLM 17 X P.S.201	ARS, Yellamanchili
23	YLM-146	G.Til -2 × Vinayak	ARS, Yellamanchili
24	Madhavi	Selection from local germplasm	ARS, Yellamanchili
25	G.Till-4	G.Til -1 × RT-125	ARS, Amreli

Cont..

Table 3.1 cont..

S.No	Genotype	Pedigree	Source
26	G.Til-5	AT-90 × AT-104	ARS, Amreli
27	G.Til-6	AT-117 × G.Til -2	ARS, Amreli
28	G.Til-10	Selection from TNAU-17	ARS, Amreli
29	RT-103	C-7 × A6-5	RRS, Mandor
30	Nirmala	Mutant of B-67	OUAT, Bhubaneswar
31	Swetha Til	E-8 × IS-113	RARS, Jagtial
32	Rajeswari	Selection from N-62-39	RARS, Jagtial
33	TKG-22	HT-6 × JLT-3	ZARS, Tikamgarh
34	TKG-55	TC-25 × TNAU-10	ZARS, Tikamgarh
35	TKG-306	CST-785 × TKG-22	ZARS, Tikamgarh
36	TKG-308	JLT-26 × JLT-7	ZARS, Tikamgarh
37	E-8	Selection from local material of northern karnataka	UAS, Dharwad
38	JCS-1020	E-8 × IS-113	RARS, Jagtial
39	JCS-3603	Hima × SI-2584	RARS, Jagtial
40	TMV-4	Selection from variety Sattur local of Tamil Nadu	ORS, Tindivanam
41	TMV-7	Derived from the cross Si 250 × Es 22	ORS, Tindivanam
42	VRI-4	VRI-2 × G.Til -10	RRS,Vridhachalam
43	Paiyur-1	SI-2511 × SI-2314	RRS, Paiyur

3.3.2 Crop Husbandry

The crop was provided with fertilizers 40:20:20 N:P:K kg ha^{-1}. Half of N and entire P and K were applied as basal dose and second half of N was applied as top dressing after 35 days of sowing. The crop was raised under completely irrigated conditions. All the recommended cultural and agronomic measures were followed during the crop period.

3.4 DATA RECORDING

3.4.1 Morphological Characterization

Twenty morphological traits on ten randomly tagged plants from each entry in each replication were recorded as per National test guidelines for Distinctness, Uniformity and Stability (DUS) testing given by Protection of Plant Varieties and Farmers' Rights Authority (PPV & FRA, 2001). For the assessment of DUS, the characteristics and their states as given in Table 3.2 were used.

Distinctness means a variety should be clearly distinguishable by one or more essential characteristics from any other existing variety. The variety should be sufficiently uniform in its essential characteristics, having regard to the specific features of its propagation. The variety is said to be stable if its relevant characteristics remain unchanged after repeated propagation.

DUS characteristics are considered to be most reliable in distinguishing or discriminating genotypes/ varieties. In the present investigation the DUS descriptors suggested by Protection of Plant Varieties and Farmers' Rights Authority (PPV & FRA) are used.

The data on 20 DUS descriptors were recorded from ten randomly selected representative plants in a plot of each genotype (except for days to 50 % flowering and days to maturity which were recorded on plot basis) and description of the DUS descriptors studied were described here under.

Plate 1: Overall view of experimental field

Table 3.2 DUS Characteristics for sesame genotypes studied

S. No.	Characteristics	Descriptor State	Note	Stage of observation	Type of assessment
1	2	3	4	6	7
1.	Time of flowering: Days to 50% Flowering	Early (<36) Medium (36-45) Late (>45)	3 5 7	45	VG
2.	Flower: Petal colour	White Light purple Dark purple	1 2 3	45	VS
3.	Flower: Petal hairiness	Absent Sparse Dense	1 3 5	45	VS
4.	Plant: Height of main stem (cm)	Short (<75) Medium (75-125) Tall (>125)	3 5 7	65	MS
5.	Plant: Branching	Absent Few (1-2) Medium (2.1-4.0) Profuse branching (>4)	1 3 5 7	65	VS
6.	Plant: Branching pattern	Basal branching Top branching	1 2	65	VS
7.	Stem: Hairiness	Absent Sparse Dense	1 3 5	65	VS
8.	Leaf: Lobes	Slightly lobed Deeply lobed	1 2	65	VG
9.	Leaf: Size	Small Medium Large	3 5 7	65	VG
10.	Leaf: Serration of margin	Weak Strong	3 5	65	VG
11.	Capsule: Hairiness	Absent Sparse Dense	1 3 5	75	VS
12.	Capsule: Locule number/ capsule	Four Six Eight	3 5 7	75	VS
13.	Capsule: Shape	Tapered Narrow oblong Broad oblong Square	1 2 3 4	100	VG

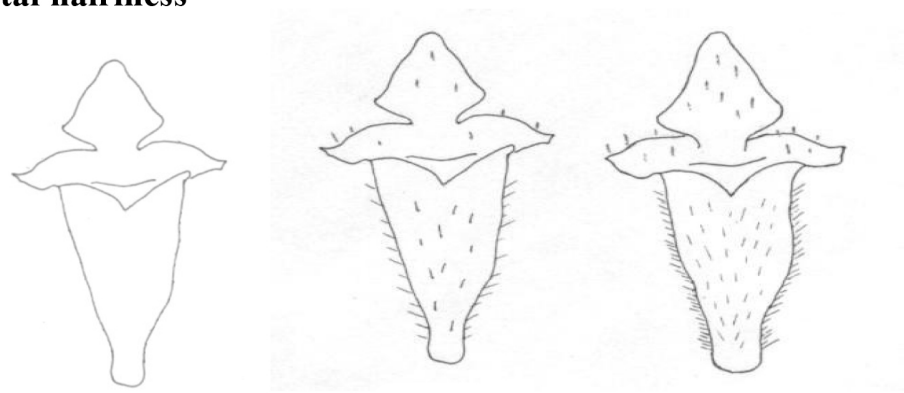
Cont..

Table 3.2 cont....

S. No.	Characteristics	Descriptor State	Note	Stage of observation	Type of assessment
1	**2**	**3**	**4**	**6**	**7**
14.	Capsule: Number / leaf axil	One More than one	1 9	100	VG
15.	Capsule: Arrangement	Alternate Opposite Cluster	1 2 3	100	VG
16.	Capsule:Length (cm)	Short (<1.5) Medium (1.5-2.5) Long (>2.5)	3 5 7	100	MS
17.	Maturity: Days to maturity	Early (<75) Medium (76-85) Late (86-95) Very late (>95)	3 5 7 9	100	VG
18.	Seed: Coat colour	White Grey Light brown Dark Brown Black	1 2 3 4 5	100	VS
19.	Seed: 1000 seeds weight (g)	Low (<2.5) Medium (2.5 -3.0) High (3.1 – 3.5) Very high (>3.5)	3 5 7 9	100	MG
20.	Seed Oil: Content (%)	Low (<45) Medium (45-50) High (> 50)	3 5 7	100	MG

MG: Measurement by single observation of a group of plants or parts of plants
MS: Measurement of a number of individual plants or parts of plants
VG: Visual assessment by a single observation of a group of plants or parts of plants
VS: Visual assessment by observation of individual plants or parts of plants

Flower: Petal hairiness

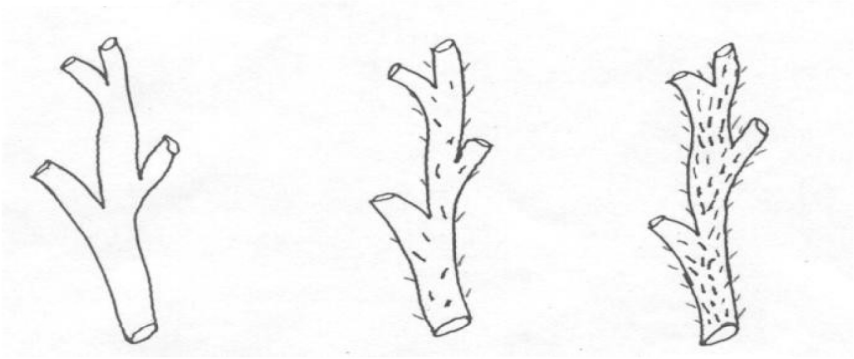


1
Absent

3
Sparse

5
Dense

Stem: Hairiness

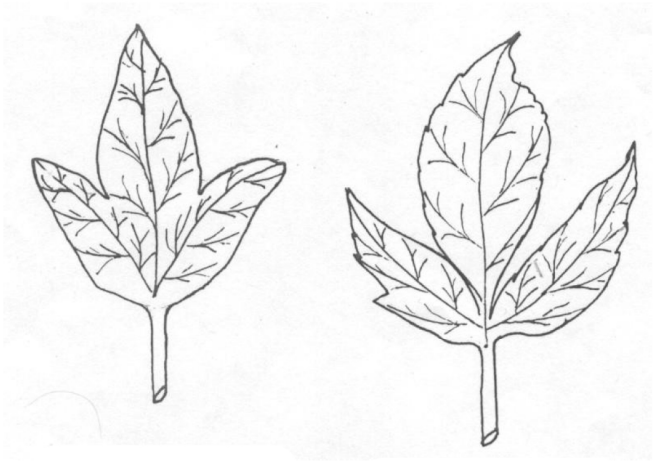


1
Absent

3
Sparse

5
Dense

Leaf: Lobes

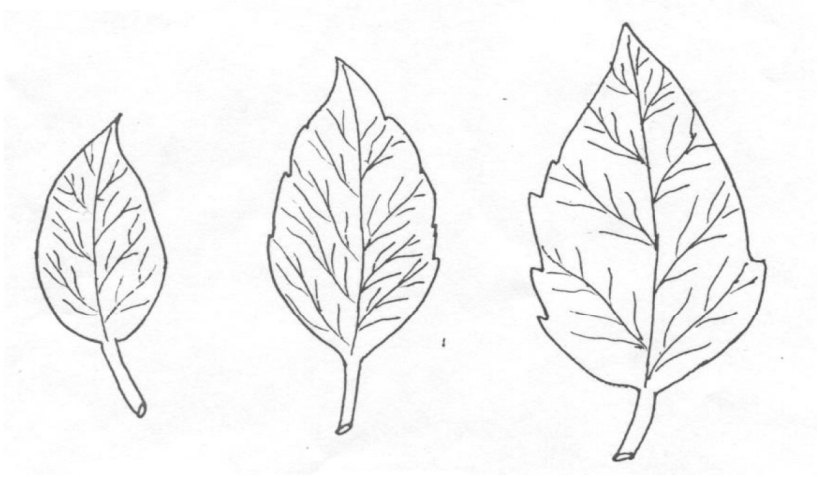


1
Slightly lobed

2
Deeply lobed

Fig. 3.1. Pictorial depiction of DUS characters

Leaf: Size

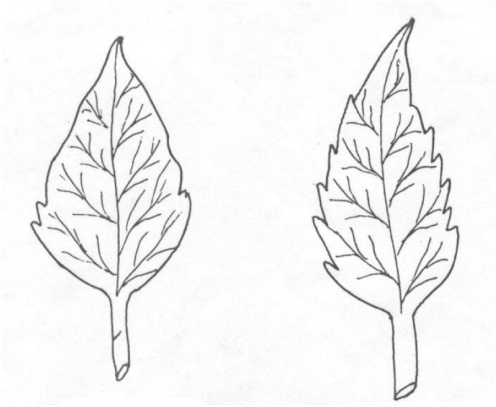


3
Small

5
Medium

7
Large

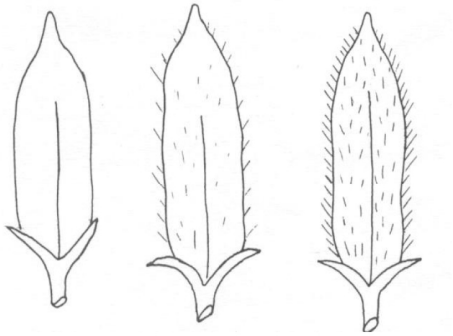
Leaf: Serration of margin



3
Weak

5
Strong

Capsule: Hairiness



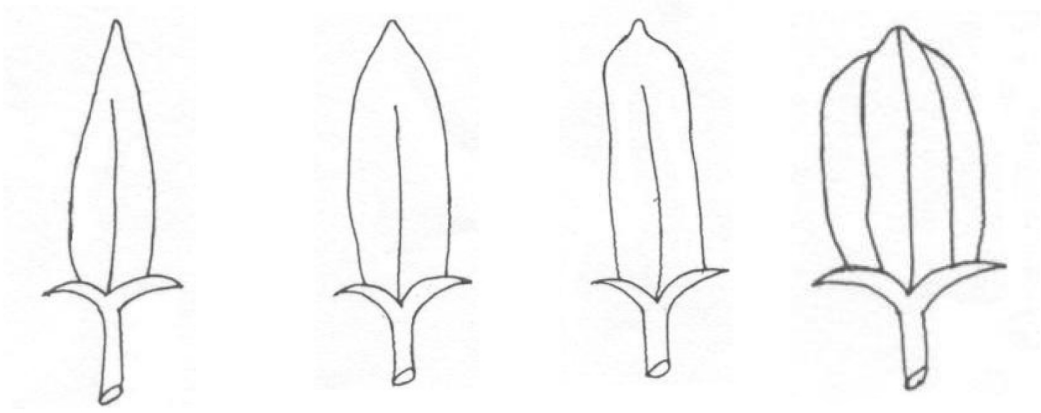
1
Absent

3
Sparse

5
Dense

Fig. 3.1. Pictorial depiction of DUS characters

Capsule: Shape



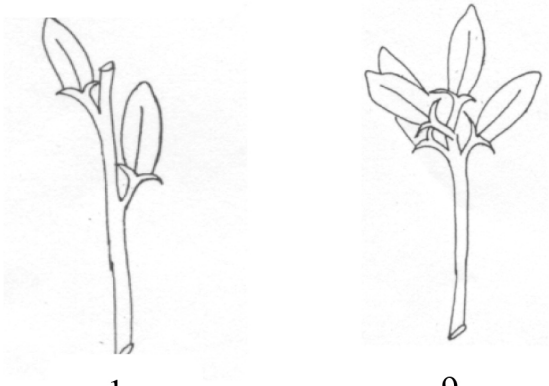
1
Tapered

2
Narrow oblong

3
Broad oblong

4
Square

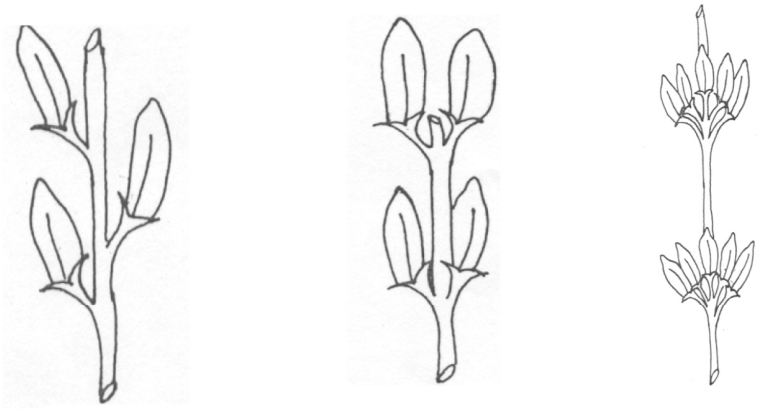
Capsule: Number per leaf axil



1
One

9
More than one

Capsule: Arrangement



1
Alternate

2
Opposite

3
Cluster

Fig. 3.1. Pictorial depiction of DUS characters

3.4.1.1 Days to 50 per cent flowering

Number of days taken from the date of sowing to the opening of flowers in 50 percent of plant population in each genotype was recorded and the genotypes were grouped as early, medium and late flowering types.

Category	Number of days
Early	<36
Medium	36-45
Late	>45

3.4.1.2 Fower petal colour

The flower petal colour was recorded on visual assessment based on group of plants at peak flowering stage and the genotypes were grouped as white, light purple and dark purple.

3.4.1.3 Flower petal hairiness

The flower petal hairiness was recorded on visual assessment at peak flowering stage on fully opened fresh flowers and the genotypes were grouped as absent, sparse and dense hairiness types.

3.4.1.4 Plant height (cm)

Plant height at the time of harvest was measured from the base of plant at ground level to the tip of the plant in centimeters and the genotypes were grouped as short, medium and tall types.

Category	Height(cm)
short	< 75
Medium	75 – 125
Tall	> 125

3.4.1.5 Plant branching

Total number of branches originated from the main shoot from each selected plant was recorded and the genotypes were grouped as absent, few, medium and profuse branching.

Category	No. of branches
Absent	0
Few	1.0 - 2.0
Medium	2.1 - 4.0
Profuse branching	> 4.0

3.4.1.6 Plant branching pattern

Plant branching pattern was recorded at the time of harvest based on visual assessment, whether the branches aroused from top portion or basal portion of the main shoot and the genotypes were grouped as basal branching and top branching.

3.4.1.7 Stem hairiness

The hairiness of the stem was recorded on visual assessment of plants at peak flowering stage and the genotypes were grouped as absent, sparse and dense.

3.4.1.8 Leaf lobes

The leaf lobes were recorded by visual assessment of fully developed 8th leaf and the genotypes were grouped as slightly lobed and deeply lobed.

3.4.1.9 Leaf size

Fully developed two alternate middle leaves from ten plants were used to measure the leaf size and the genotypes were grouped into small, medium and large types.

3.4.1.10 Leaf serration of margin

Leaf serration of margin was recorded by visual assessment of plants at complete flowering stage and the genotypes were grouped as weak and strong serration of margin types.

3.4.1.11 Capsule hairiness

The capsule hairiness was recorded by observation of plants at complete capsule formation stage and the genotypes were grouped as absent, sparse and dense types.

3.4.1.12 Locule number per capsule

The number of locules per capsule was counted and recorded at complete capsule formation stage and genotypes were grouped as four, six and eight loculed types.

3.4.1.13 Capsule shape

Capsule shape was recorded by visual observation of fully developed capsules from ten plants and were grouped as tapered, narrow, oblong, broad oblong and square.

3.4.1.14 Capsules number per leaf axil

The number of capsules per leaf axil was counted and recorded at maturity stage and the genotypes were grouped as one and more than one capsule per leaf axil.

3.4.1.15 Capsule arrangement

The capsule arrangement was recorded by visual assessment of plants at maturity stage and the genotypes were grouped as alternate, opposite and cluster types.

3.4.1.16 Capsule length (cm)

The length of capsule from base to tip was measured in centimeters with vernier calipers and the mean length of capsules was recorded. Based on the length, the genotypes were grouped as short, medium and long capsule types.

Category	Length (cm)
Short	<1.5
Medium	1.5-2.5
Long	>2.5

3.4.1.17 Days to maturity

Number of days taken to maturity of each genotype was calculated from the date of sowing to physiological maturity and the genotypes were grouped as early, medium, late and very late maturity types.

Category	Days
Early	< 75
Medium	76 – 85
Late	86 – 95
Very late	>95

3.4.1.18 Seed coat colour

The seed coat colour of each genotype was observed under natural day light condition and genotypes were grouped as white, grey, light brown, dark brown and black seed coloured types.

3.4.1.19 Thousand seed weight (g)

One thousand seeds were taken randomly from sampled plants and their weightswere measured with the help of electronic top pan balance and expressed in grams and the genotypes were grouped as low, medium, high and very high.

Category	Weight (gm)
Low	2.5
Medium	2.5 - 3.0
High	3.1 - 3.5
Very high	> 3.5

3.4.1.20 Oil content (%)

Fully developed and dried healthy seeds were sampled and oil percentage was assessed through NMR Spectroscopy and the genotypes were grouped into low, medium and high.

Category	Oil content (%)
Low	<45
Medium	45-50
High	>50

3.4.2 Quantitative Traits

Observations were also recorded on five randomly selected competitive plants for other quantitative traits *viz.,* number of primary branches per plant, number of capsules per plant, number of seeds per capsule and seed yield per plant. The details of the data recorded were as follows.

3.4.2.1 Number of primary branches per plant

Total number of branches originated from the main shoot in each plant was recorded.

3.4.2.2 Number of capsules per plant

Total number of capsules per plant was counted from randomly selected plants in each genotype.

3.4.2.3 Number of seeds per capsule

Seeds of capsules at various heights were harvested when seeds were intact from each sample and the mean number of seeds per capsule was recorded.

3.4.2.4 Seed yield per plant (g)

Five randomly selected plants were harvested and threshed separately. These were cleaned, weighed and mean seed yield per plant was recorded.

3.5 STATISTICAL ANALYSIS

The treatment means for each character over three replications were subjected to the following statistical analysis. The statistical package used was INDOSTAT.

1. Analysis of variance.

2. Estimation of variability and genetic parameters

3. Estimation of genetic divergence using Mahalanobis's D^2 analysis.

3.5.1 Analysis of Variance

The data collected on individual characters were subjected to method of analysis of variance commonly applicable to randomized block design as per mathematical model proposed by Panse and Sukhatme (1961).

$$Y_{ij} = \mu + g_i + \gamma_j + e_{ij}$$

where,

Y_{ij} = Phenotypic observation on 'i'th genotype in 'j'th replication.

μ = General mean

g_i = Effect of i^{th} genotype

γ_j = Effect of j^{th} replication

e_{ij} = Random error associated with i^{th} genotype in j^{th} replication.

The analysis of variance for each character was carried out as follows:

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	Expected Mean sum of squares	F ratio
Replications	(r-1)	RSS	Mr	-	Mr/Me
Genotypes	(t-1)	VSS	Mt	$\sigma_e^2 + r\sigma_g^2$	Mt/Me
Error	(r-1) (t-1)	ESS	Me	σ_e^2	-
Total	(rt-1)	TSS			

where,

r = Number of replications

t = Number of genotypes

Mr = Mean sum of squares due to replications

Mt = Mean sum of squares due to genotypes

Me = Mean sum of squares due to error.

The significance test was carried out by referring to standard 'F' table values given by Fisher and Yates (1963).

3.5.2 Estimation of Genetic Parameters

3.5.2.1 Variance

The genotypic and phenotypic variances were calculated as per the formulae proposed by Burton (1952)

$$\text{Genotypic variance } (\sigma_e^2) = \frac{\text{MSS due to genotypes - MSS due to error}}{\text{Number of replications}}$$

$$\text{Phenotypic variance } (\sigma_p^2) = \sigma_g^2 + \sigma_e^2$$

σ_g^2 = Genotypic variance

σ_e^2 = Error variance

3.5.2.2 Genotypic and phenotypic coefficient of variation

The genotypic (GCV) and phenotypic (PCV) coefficient of variation were computed by the formulae given by Burton (1952).

$$\text{GCV (\%)} = \frac{\sigma_g}{\overline{X}} \times 100$$

$$\text{PCV (\%)} = \frac{\sigma_p}{\overline{X}} \times 100$$

where,

σ_g, σ_p and $\overline{X}$ were genotypic standard deviation, phenotypic standard deviation and general mean of the character, respectively.

Categorization of the range of variation was done as proposed by Sivasubramanian and Madhavamenon (1973)

Less than 10 %	-	Low
10 – 20 %	-	Moderate
More than 20 %	-	High

3.5.2.3 Broad sense heritability

The proportion of genotypic variance to the total variance of the population is referred to as heritability in broad sense [$h^2_{(b)}$] and was calculated by the formula given by Lush (1940).

$$\text{Broad sense Heritability} = \frac{\sigma^2_g}{\sigma^2_p} \times 100$$

where,

$h^2_{(b)}$ = Heritability in broad sense

σ^2_g = Genotypic variance

σ^2_p = Phenotypic variance ($\sigma^2_g + \sigma^2_e$)

σ^2_e = Environmental variance

As suggested by Johnson *et al.* (1955), heritability estimates were categorized as

Less than 30 %	-	Low
30 – 60 %	-	Moderate
More than 60 %	-	High

3.5.2.4 Genetic advance

Genetic advance refers to the expected genetic gain or improvement in the next generation by selecting the superior individuals under certain amount of selection pressure. From the heritability estimates, the genetic advance was estimated by the following formula given by Johnson *et al.* (1955).

$$GA = K\ (\sigma_p)\ h^2_{(b)}$$

where,

GA = Genetic advance

σ_p = Phenotypic standard deviation

$h^2_{(bs)}$ = Heritability (broad sense)

K = Selection differential at 5% selection intensity (2.06)

3.5.2.5 Genetic advance as percent of mean (GAM)

Genetic advance as percent of mean was calculated as per the formula.

$$GAM = \frac{GA}{\overline{X}} \times 100$$

where,

GA = Genetic advance

$\overline{X}$ = Grand mean of the character

The range of genetic advance as percent of mean was classified as suggested by Johnson *et al.* (1955).

Less than 10% - Low

10 – 20 % - Moderate

More than 20 % - High

3.5.3 Genetic Divergence Analysis

3.5.3.1 Mahalanobis' D^2 analysis

The data collected on different characters was analyzed using Mahalanobis's D^2 analysis to determine the genetic divergence among the genotypes.

3.5.3.1.1 Test of significance

Variances were calculated for all the characters investigated and test of significance was done. Analysis of covariance for the character pairs was estimated on the basis of mean values (Panse and Sukhatme, 1961). After testing the difference between genotypes for each of the characters, a simultaneous test of significance for differences in the mean values of a number of correlated variables with regard to the pooled effect of characters was carried out using 'V' statistic, which in turn utilizes Wilk's criterion. The sum of squares and sum of products of error and error + variety, variance – covariance matrix were used for this purpose.

The estimation of Wilk's criterion was done using the following relationship.

$$\text{`}\Lambda\text{'} = \frac{|E|}{|E+V|}$$

where,

^ = Wilk's criterion

(E) = Determinant of error matrix and

(E+V) = Determinant of error + variety matrix

The significance of ' Λ ' was tested by

$$\chi^2_{pq} = V = - m \log_e \Lambda$$

where,

m = n - (p + q + 1)/2 with 'pq' degrees of freedom

n = Degrees of freedom of error + varieties

p = Number of characters

q = Number of genotypes – 1

$\log_e \Lambda$ = 2.3407 $\log_{10} \Lambda$

V (Stat) is distributed as χ^2 with pq degrees of freedom.

3.5.3.1.2 Transformation of correlated variables

Transformation was done using pivotal condensation method. Transformation of correlated variables into standardized uncorrelated ones was done before working out the D^2 values because computation of D^2 values was reduced to simple enumeration of differences in mean values of various characters of the two genotypes i.e., Σdi^2.

3.5.3.1.3 Computation of D^2 values

The D^2 value between 'i^{th}' and 'j^{th}' genotypes for 'p' characters was calculated as

$$D_{ij}^2 = p\Sigma_{t=1} (\overline{Y_{it}} - \overline{Y_{jt}})^2$$

where,

$\overline{Y_{it}}$ = uncorrelated mean value of i^{th} genotype for character 't'

$\overline{Y_{jt}}$ = uncorrelated mean value of j^{th} genotype for character 't'

D_{ij}^2 = D^2 between i^{th} and j^{th} genotype.

3.5.3.1.4 Testing the significance of D^2 values

The D^2 value obtained for a pair of genotypes is taken as calculated value of χ^2 and is tested against the tabulated value of χ^2 for p degrees of freedom where 'p' is the number of characters considered.

3.5.3.1.5 Grouping of genotypes into various clusters

The grouping of genotypes into different clusters was done using the Tocher's method as described by Rao (1952). The criterion was that, two varieties belonging to the same cluster at least on an average show a smaller D^2 value than those belonging to different clusters. For this purpose D^2 values of all combinations of each genotype were arranged in ascending order of magnitude in a tabular form as described by Singh and Choudhary (1977).

To start with two genotypes having the closest distance from each other were considered, to which the third genotype having the smallest D^2 value from the first two genotypes was considered and so on. Similarly, the next nearest fourth population was considered and this procedure was continued. At certain stage when it was felt that after adding a particular genotype there was an abrupt increase in the average D^2, that the genotype was not considered for including in that cluster. The genotypes of the first cluster were then eliminated and the rest were treated in a similar way. This procedure was continued till all the genotypes were included into one or other cluster.

3.5.3.1.6 Average intra cluster distance

For the measurement of intra cluster distances, the formula used was

$$\frac{\sum D_i^2}{n}$$

where,

$\sum D_i^2$ = the sum of distances between all possible combinations (n) of populations included in a cluster.

3.5.3.1.7 Average inter cluster distance

Clusters were taken one by one and the distances from other clusters were calculated. The distance between two clusters was the sum of D^2 values between the members of one cluster to each of the members of the other clusters divided by the product of number of genotypes in both the clusters under consideration.

$$\text{Average inter cluster distance} = \frac{D^2}{(n_1 \times n_2)}$$

where, n_1 and n_2 are number of genotypes of two clusters.

3.5.3.1.8 Cluster diagram

The clusters and their mutual relationships were presented diagrammatically. The square root of average D^2, which was an approximate measure of divergence between groups, had been used to denote the distance.

3.5.3.1.9 Contribution of individual characters towards divergence

In all combinations, each character was ranked on the basis of their contribution towards divergence between two entries ($d_i = Y_{it} - Y_{jt}$). Rank 1 is given to the highest mean difference and the rank P to the lowest difference, where, P is the total number of characters. Percentage contribution of each character (X) towards genetic divergence was calculated using the following formula.

$$\text{Percentage contribution of the character(X)} = \frac{(N \times 100)}{M}$$

where,

N = Number of genotype combinations where the character was ranked first

M = All possible combinations of number of genotypic pairs

3.6 MOLECULAR CHARACTERIZATION OF GENOTYPES USING SSR MARKERS

3.6.1 List of Chemicals Used

Chemicals used in the study are given in the appendix A.

3.6.2 Preparation of Stocks and Buffer Solutions

Procedure for the preparation of stocks and buffer solutions used in the present study are given in the appendix B.

3.6.3 List of Equipments Used

Equipments used in the study are given in the appendix C.

3.6.4 Germination of Sesame Seeds in Petriplates

Blotting paper was kept in petri plate and it was labeled on the outside top and bottom portion with ID assigned to that particular genotype. From each genotype 20-30 seeds were selected and kept for germination in petriplates with blotting paper in a dark room with regular showering up to one week. These one week age seedlings were used for DNA extraction.

3.6.5 Isolation of Genomic DNA

Genomic DNA was extracted from one week old seedlings using the modified Cetyl Tri Methyl Ammonium Bromide (CTAB) method (Porebski *et al.*, 1997) with some modifications. The modifications were made to improve the quality of the DNA.

- The sample was cut into pieces and 1ml of pre warmed CTAB extraction buffer was added and grinded with pestle and mortar.
- The grinded sample was transferred into a sterile 15ml eppendorf tube and added 5ml of 60^0 C extraction buffer and 50mg Poly Vinyl Pyrrolidone (PVP).
- The solution was vortexed for 30-40 seconds and the tubes were incubated at 60^0C in a water bath for 60 minutes with 2-3 times occasional swirling during incubation. The tubers were brought to room temperature.

- 6ml of Chloroform and Isoamylalchol (CIA) in the ratio of 24:1 was added to 15ml tubes and mixed thoroughly.
- Tubes were centrifuged @ 3000 rpm for 20 minutes at room temperature.
- The aqueous supernatant solution was pipetted out and transferred to a fresh tube for DNA isolation. CIA extraction step was repeated to remove PVP.
- 2.5M NaCl was added to final aqueous solution and mixed well.
- Two volumes of cold (-20^{0}C) 95% ethanol was added and mixed. Then tubes were placed in freezer (-20^{0}c) for 10 minutes to accelerate the precipitation. Solution left @ 4-6^0 C to precipitate overnight.
- The supernatant was poured off and the pellet was washed with cold 70% ethanol (0-4^{0}C).
- Pellet was dried at 37^{0}C in an oven or vacuum until dry (one hour).
- The pellet was dissolved in 300µl of TE buffer overnight @ 4-6 ^{0}C and transferred to 1.5 ml eppendorf tube.
- 3µl RNase-A (10mg/ml) was added to the DNA dissolved tube and incubated at37^{0}C in water bath for 1 hour.
- 3µl protienase K (10mg/ml) was also added and incubated at 37^{0}C in water bathfor 15-30 minutes.
- 150µl of phenol and 150 µl of chloroform were added to eppendorf tubes and vortexed.
- Microcentrifugation was performed at 14000 rpm for 10-15mins.
- Upper layer was collected in 1.5ml tube and 50µl TE was added to phenol phase. To this, 1/10th volume of 2M sodium acetate and 2 volumes of absolute ethanol were added and mixed. The tubes were kept overnight at -80^{0}C.
- Centrifugation of the tubes at 14000 rpm for 10-20 minutes was done.
- The pellet was washed with 70% ethanol and added 100-200 µl of TE for the DNA sample.

3.6.6 Assessment of Quality and Quantity of DNA

Quality and quantity assessment of DNA can be done by three different methods *viz.*, Spectrophotometry, Agarose gel electrophoresis and by using Nanodrop. In the present study DNA quality and quantity were assessed by using Agarose gel electrophoresis (AA Hoefer Electrophoresis unit, Richmond, CA 94804, USA) and Nanodrop (Thermo Scientific 2000) respectively.

3.6.6.1 Quantification of DNA by 0.8% agarose gel electrophoresis

Agarose of 0.8g was placed in conical flask containing 100ml 1X TBE buffer. The conical flask along with its contents was placed in microwave oven until agarose get melted completely and clear solution was formed. The flask was took out from the oven and allowed the solution to cool until it reaches 50-55^{0}C. 3µl of Ethidium Bromide (10 mg ml^{-1}) was added to 100ml of agarose gel and mixed it thoroughly. Later this solution was poured slowly into the gel casting tray which is preset with 0.5 mm combs, to avoid the formation of bubbles. After solidifying the comb was removed gently from the gel and then placed the gel with casting tray in gel tank.

3.6.6.2 Electrophoresis of the DNA samples

3 µl of each of dissolved genomic DNA samples were mixed with 2 µl of 6X Gel loading dye (40% sucrose and 0.25% bromo phenol blue) and were loaded onto 0.8 % agarose-1X TBE gel. Then, the gel with loaded samples was electrophoresed at 100V at room temperature for about an hour. After that, the gel was visualized in anUV gel documentation system (SYNGENE G Box) and saved the image for further use. Later, the intensity and thickness of genomic DNA was compared to λ DNA and the concentration of DNA in individual samples was determined.

3.6.6.3 Quantification of DNA by Nanodrop method

Nanodrop (Thermo Scientific 2000) was used to assess the quantity and quality of DNA employing following procedure.

- Before initializing the Nanodrop reader, the pedestal was cleaned with tissue paper to remove dust particles.
- Then for initializing the instrument, 1µl of distilled water was placed on the pedestal and clicked on measure option. After initialization, the pedestal was cleaned with tissue paper and placed 1µl of 1X TE buffer on pedestal for blank measurement and the pedestal was cleaned with tissue paper. 1µl of DNA sample was placed on pedestal to measure the quantity and quality of DNA.

The ratio of absorbance at 260 nm and 280 nm was used to assess the purity of DNA. A ratio of ~1.8 is generally accepted as "pure" for DNA. If the ratio is appreciably lower in either case, it may indicate the presence of protein, phenol or other contaminants that absorb strongly at/or near 280 nm. After quantification DNA samples were diluted to a working concentration of 30ng/µl.

3.6.7 Amplification of DNA Using Polymerase Chain Reaction

DNA was subjected to Polymerase Chain Reaction (PCR) by using 34 SSR (Simple sequence Repeats) markers reported in earlier studies having PIC values morethan 0.5 and are presented in Table 3.3.

PCR tubes of 0.2ml were taken and 2 µl of DNA (50ng/µl) was added. PCR reaction was performed in a 10µl volume of mix containing the components as in Table 3.3.

Table 3.3 PCR mixture, components concentration and volume used for reaction

S. No.	PCR Component	Concentration	Volume
1	Genomic DNA	50ng/µl	2 µl
2	Forward Primer	10pM/ µl	0.5µl
3	Reverse Primer	10pM/ µl	0.5µl
4	Taq Buffer with $MgCl_2$	10X	1.0 µl
5	DNTPs	2.5Mm	0.5 µl
6	Taq DNA polymerase enzyme	5U/µl	0.1µl
7	Sterile distilled water	-	5.4µl
	Total	**-**	**10µl**

The steps from 2-4 were repeated for 35 times for amplification of targeted DNA. Annealing temperature of each primer was standardized by doing PCR with the temperature range of 54-64^{0}C. The reaction mixture was given a short spin for thorough mixing of the components and the PCR tubes were placed in the gradient thermal cycler(Agilent Sure cycler 8800).The PCR products were stored at 4^{0}C for short periods and at -20^{0}C for long duration. The amplification conditions are presented below. List of primers used in this study are presented in Table 3.4.

S. No.	Step	Temperature (^{0}C)	Duration	Cycles
1	Denaturation	94	5 mins	1
2	Denaturation	94	1 min	35
3	Annealing	52-64	45 sec	
4	Extension	72	1 min	
5	Final extension	72	10 mins	1
6	Hold	4	∞	

Table 3.4 List of primers used in molecular diversity study in Sesame

S.No.	Primer name	Forward primer sequence	Reverse primer sequence	References
1	GBssr-sa-05	TCATATATAAAAGGAGCCCAAC	GTCATCGCTTCTCTCTTCTTC	Zhang *et al.*(2012)
2	GBssr-sa-08	GGAGAAATTTTCAGAGAGAAAAA	ATTGCTCTGCCTACAAATAAAA	
3	GBssr-sa-33	TTTTCCTGAATGGCATAGTT	GCCCAATTTGTCTATCTCCT	
4	GBssr-sa-182	CCATTGAAAACTGCACACAA	TCCACACACAGAGAGCCC	
5	SSR 19	CTCATCTACCCACACCATCTA	CACCAATTCTTTTGTGTCTT	Gogoi *et al.*(2018)
6	SSR 33	ACAATCGTAGTCCTTTCTTGA	GCAAAGGTTGTTGTTGTCTC	
7	SM-10-128	CCAACACTCATTGGCTCAGA	GACCTGCCAAAATGACATCC	
8	SM-10-134	GGCCTAGCTAGCAGAATCACTG	ATAATCCAAGTGGCGAGCAT	
9	SM-10-141	GGCCTAGCTAGCAGAATCACTG	ATAATCCAAGTGGCGAGCAT	
10	SSR211	CCTGTAGGTGCCAAGATGGT	GCATGTCGGAATTAAAACTCG	
11	SSR212	ACAGAGAGAGGTGGGAAATC	AGCAAGAAAACAGAAACAGC	
12	SSR215	GTTGAGGTGGTAGAATGGAA	GAGAGCATCAACGTCAAAAC	
13	SSR217	AAGCATAAGCCAGAAAACAC	GCTTTCCGTTCATATAATGC	
14	SSR223	GCATTGTAAAAATGGAAACG	GTTTTGCACGGCTTTATTAC	
15	SSR224	TAGACGTTCCACTTGTCCTC	TCCTCCACCAAATGATAAAC	Surapaneni *et al.* (2014)
16	SM-10-153	GGGAGCCTTATTTCTGTCTTCA	TAGCTCAATACCAGGAGCAAAA	
17	SM-10-173	CGAGAAAGGACTCCGCATTA	GCAGAATCCGCTCTTTTCTG	

Cont…

Table 3.4. Cont…

S.No.	Primer name	Forward primer sequence	Reverse primer sequence	References
18	SM-10-185	CACCAGGGTTGTTTGTGTGT	GTATTTCGCAGACCCTGAGC	
19	Si-3	AATTACCGGCCTCTCAGCTT	TGCCTCTGCAACTGTTTGAC	
20	Si-14	GGAGACACACAACCGCACTA	GCGGCAGAAGGTTCAGATAA	
21	Si-15	TATAGGGCCCGACTTATCCA	CACATCTTTTATCCCATTCAACA	
22	Si-34	CAGGCGTAAAGCCTCTCTGT	TGTGGAAAAAGTGCCTCCTC	
23	SM-10-254	TGCTTTTCCTCTCCAAATTCA	GACTCAACTGCCCCAGATTC	
24	SM-10-288	CATAGGTGACAAGATGTACTGC	TTAACTCTGCTTTCCTACCACT	
25	SM-10-301	AGTTGAAGTGTGGAAGAAGG	GTGTAGCACAAGACCAAATAGA	
26	SM-10-331	ATTAGAGCAGAAGAACGGAGTA	GTTTGATTTAGCCGATTGAG	
27	SM-10-355	GTAGTGAACAGGAAAGAAGGG	ATGAGTGAGTTTAGTGCAGCTT	
28	SM-10-404	CTTTCTTCAGTCCAATCTTCTC	GGATGACAATTTCTTACTAGCC	
29	SEM-12-14	ACTAGCCATAGACATAGGCAAA	AGATGAGAGTGGTGCAGTTAAT	
30	SEM-12-26	ATAAGACTGCGAAAACCCTCAA	ATGCTGATAATGGCAGACAGAA	
31	SEM-12-58	GAATCCTCGATAACCAAACTGC	AAGGGACCTCAACCATAACCTT	
32	CUSSR 10	AGAGATGGAGCTTGAACTAGG	TGTTTTTGGGTGCTACTACTC	Iqbal *et al*. (2016)
33	CUSSR 16	TTGTGGATTGTAAGCTATTCC	GTGACAATTCTTGCTCGTAAT	
34	CUSSR 178	GCCCACCCATAGAAAGAAAA	TTCTGCCCTAACCTCTCAACTC	

3.6.7.1 Resolution of PCR Products

The PCR product was loaded on to the 3.5% agarose gel using Hoefer Electrophoresis unit (Richmond, CA 94804, USA) by mixing with 2 µl of 6X loading dye. A 100 bp ladder was loaded as a reference marker. The gel was run at constant voltage of 100V for about 2-3 hours, until the ladder got properly resolved. The banding pattern was analyzed using gel documentation system (SYNGENE G Box).

3.6.8 Data Scoring and Analysis

The amplified products for marker analysis were scored visually based on the presence (taken as '1') or absence (taken as '0') of band for each primer. Each marker fragment was treated as a unit character and only clear and unambiguous bands were scored. Genetic diversity parameters like number of alleles per locus and major allele frequency were calculated by using markers data. The allele frequency represents the frequency of a particular allele for each marker in the population. Polymorphic information content (PIC) was calculated using the following formula (Hwang *et al*., 2009, Barik *et al*., 2019):

$$PIC = 1-\Sigma(Pij)^2$$

Where,

Pij- the frequency of the i^{th} pattern revealed by the j^{th} primer summed across all patterns revealed by the primers.

The Jaccard's coefficients were used to construct a dendrogram using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) in DARwin software version 6.0.21 (Perrier,2006). For accessions being used in this study, QR (Quick Response) codes were generated using online tool (available at www.barcode-generator.org).

Chapter - IV

Results & Discussion

Chapter IV

RESULTS AND DISCUSSION

The present study on **"DUS characterization and molecular diversity analysis in Sesame (*Sesamum indicum* L.)"** was conducted during *Rabi,*2022. The experimental results from the above investigation were presented here under the following sub-heads.

4.1 MORPHOLOGICAL CHARACTERIZATION USING DUS DESCRIPTORS

4.2 ANALYSIS OF VARIANCE

4.3 MEAN PERFORMANCE

4.4 GENETIC PARAMETERS

4.5 GENETIC DIVERGENCE

4.6 MOLECULAR DIVERSITY

4.1 MORPHOLOGICAL CHARACTERIZATION USING DUS DESCRIPTORS

4.1.1 DUS Characterization

DUS test is considered to be the foundation of plant variety protection and also to identify a new variety from reference collection (Kwon *et al.,* 2005). In any crop improvement programme, identification and characterization of new cultivars is crucial for their efficient utilization and germplasm conservation. Traditionally, morphological trait characterization has been used as a foundation for germplasm classification, visual identification, differentiation, and cataloguing. Characterization based on plant morphological characteristics have long been acknowledged as the unquestionable descriptors for DUS testing and varietal classification of crop varieties (Joshi *et al.*, 2018). It could reveal their phylogeny, which would be extremely beneficial to a plant breeder in using these germplasm in frontier area of sesame research.

The present investigation was conducted to characterize 43 sesame genotypes for 20 characters based on PPV & FR testing guidelines and results are furnished here under in Table 4.1 and Fig. 4.1.

4.1.1.1 Time of flowering: Days to 50 % flowering

Thirty six genotypes (83.72 %) showed medium duration of flowering (36-45 days), while seven genotypes (16.27 %) showed early duration of flowering(<36days). Medium duration flowering was predominant over early duration. Similar results were reported by Ukani (2018), Savaliya (2021), Mathur *et al.* (2022).

4.1.1.2 Flower: Petal colour

The corolla colour of the flower is one of the important characters for characterization. Twenty seven accessions exhibited light purple petal colour (62.79 %) while white colour by 16 genotypes (37.20 %). This result was in agreement with the findings of Suhasini *et al.* (2006), Mahesh (2014), Singh *et al.* (2017), Bhoot *et al.* (2019), Palakshappa *et al.* (2020), Mathur *et al.* (2022). Genotypes Nirmala, Paiyur-1, Rajeswari, TMV-7, YLM-17 exhibited light purple petal colour more prominently.

4.1.1.3 Flower: Petal hairiness

Among 43 genotypes, 28 genotypes (65.11 %) were distinguished with dense hairiness and the remaining 15 genotypes (34.88 %) showed sparse hairiness. This result was in accordance with the findings of Ukani (2018), Savaliya (2021). Pratap, Nirmala, Hima, JCS-1020, Rajeswari recorded dense petal hairiness.

4.1.1.4 Plant: Height of main stem (cm)

For plant height among 43 genotypes, all the 43 genotypes (100.00 %) exhibited medium (75-125 cm) plant height. There is no variation observed for this trait indicating that this trait may not be useful for discrimination of the varieties. Similar findings were also reported by Ukani (2018), Bhoot *et al.* (2019).

Table 4.1 DUS characterization in 43 genotypes of sesame

S. No.	Name of the descriptor	Descriptor state	No. of accessions	Genotypes	Frequency (%)
1	Time of flowering: Days to 50% flowering	Early (<36)	7	Pratap, TKG-21, RT-125, RT-390, Swetha til, TKG-22, JCS-1020	16.27
		Medium (36-45)	36	RT-54, RT-46, JCS-2454, CUMS-17, JTS-8, RT-351, Chandana, VRI-2, RT-346, G.Til-2, Hima, RT-127, Gouri, YLM-11, YLM-17, G.Til-1, G.Til-3, YLM-66, YLM-146, Madhavi, G.Til-4, G.Til-5, G.Til-6, G.Til-10, RT-103, Nirmala, Rajeswari, TKG-55, TKG-306, TKG-308, E-8,JCS-3603, TMV 4,TMV-7,VRI-4, Paiyur-1	83.72
		Late (>45)	0	--	-
2	Flower: Petal colour	White	16	Pratap, RT-54, JCS-2454, JTS-8, RT-351, VRI-2, RT-346, G.Til-2, RT-127, Gouri, YLM-11, G.Til-1, YLM-66, YLM-146, TKG-55, TKG-308	37.20
		Light purple	27	TKG-21, RT-46, CUMS-17, Chandana, RT-125, RT-390, Hima, YLM-17, G.Til-3, Madhavi, G.Til-4, G.Til-5, G.Til-6, G.Til-10, RT-103, Nirmala, Swetha til, Rajeswari, TKG-22, TKG-306, E-8, JCS-1020, JCS-3603, TMV-4, TMV-7, VRI-4, Paiyur-1	62.79
		Dark purple	0	--	-
3	Flower: Petal hairiness	Absent	0	--	-
		Sparse	15	JCS-2454, CUMS-17, JTS-8, RT-351, VRI-2, RT-125, RT-346, G.Til-1, Madhavi, G.Til-5, G.Til-6, TKG-306, TMV-4, TMV-7, Paiyur-1	34.88
		Dense	28	Pratap, TKG-21, RT-54, RT-46, Chandana, RT-390, G.Til-2, Hima, RT-127, Gouri, YLM-11, YLM-17, G.Til-3, YLM-66, YLM-146, G.Til-4, G.Til-10, RT-103, Nirmala, Swetha til, Rajeswari, TKG-22, TKG-55, TKG-308, E-8, JCS-1020, JCS-3603, VRI-4	65.11
4	Plant: Height of main stem (cm)	Short (<75)	0	--	-
		Medium (75-125)	43	Pratap, TKG-21, RT-125,RT-390, Swetha til, TKG-22, JCS-1020, RT-54, RT-46, JCS-2454, CUMS-17, JTS-8, RT-351, Chandana, VRI-2, RT-346, G.Til-2, Hima, RT-127, Gouri, YLM-11, YLM-17, G.Til-1, G.Til-3, YLM-66, YLM-146, Madhavi, G.Til-4, G.Til-5, G.Til-6, G.Til-10, RT-103, Nirmala, Rajeswari, TKG-55, TKG-306, TKG-308, E-8,JCS-3603-TMV 4,TMV-7,VRI-4, Paiyur-1	100
		Tall (>125)	0	--	-

Cont..

Table 4.1 cont..

S. No.	Name of the descriptor	Descriptor state	No. of accessions	Genotypes	Frequency (%)
5	Plant: Branching	Absent	0	--	-
		Few (1-2)	1	JCS-1020	2.32
		Medium (2.1-3.0)	19	Pratap, TKG-21, RT-54, JCS-2454, RT-351, RT-346, RT-390, G.Til-2, RT-127, G.Til-1, G.Til-3, G.Til-4, G.Til-5, G.Til-6, Rajeswari, TKG-306, TKG-308, E-8, TMV-4	44.18
		Profuse branching (>4)	23	RT-46, CUMS-17, JTS-8, Chandana, VRI-2, RT-125, Hima, Gouri, YLM-11, YLM-17, YLM-66, YLM-146, Madhavi, G.Til-10, RT-103, Nirmala, Swetha til, TKG-22, TKG-55, JCS-3603, TMV-7, VRI-4, Paiyur-1	53.48
6	Plant: Branching pattern	Basal branching	20	TKG-21, RT-46, CUMS-17, RT-351, Chandana, VRI-2, RT-125, RT-346, G.Til-2, RT-127, YLM-11, YLM-17, Madhavi, Nirmala, TKG-22, TKG-55, TKG-308, JCS-3603, TMV-7, VRI-4, Paiyur-1	46.51
		Top branching	23	Pratap, RT-54, JCS-2454, JTS-8, RT-390, Hima, Gouri, G.Til-1, G.Til-3, YLM-66, YLM-146, G.Til-4, G.Til-5, G.Til-6, G.Til-10, RT-103, Swetha til, Rajeswari, TKG-306, E-8, JCS-1020, TMV-4	53.48
7	Stem: Hairiness	Absent	4	VRI-2, G.Til-3, TMV-4, TMV-7	9.30
		Sparse	38	Pratap, TKG-21, RT-125,RT-390, Swetha til, TKG-22, JCS-1020, RT-46, JCS-2454, CUMS-17, JTS-8, RT-351, Chandana, RT-346, G.Til-2, Hima, RT-127, Gouri, YLM-11, YLM-17, G.Til-1, YLM-66, YLM-146, Madhavi, G.Til-4, G.Til-5, G.Til-6, G.Til-10, RT-103, Nirmala, Rajeswari, TKG-55, TKG-306, TKG-308, E-8,JCS-3603, VRI-4, Paiyur-1	88.37
		Dense	1	RT-54	2.32
8	Leaf: Lobes	Slightly lobed	14	TKG-21, RT-54, RT-46, JCS-2454, RT-351, RT-125, RT-127, YLM-11, G.Til-10, RT-103, Rajeswari, TKG-22, TMV-4, Paiyur-1	32.55
		Deeply lobed	29	Pratap, CUMS-17, JTS-8, Chandana, VRI-2, RT-346, RT-390, G.Til-2, Hima, Gouri, YLM-17, G.Til-1, G.Til-3, YLM-66, YLM-146, Madhavi, G.Til-4, G.Til-5, G.Til-6, Nirmala, Swetha til, TKG-55, TKG-306, TKG-308, E-8, JCS-1020, JCS-3603, TMV-7, VRI-4	67.44

Cont..

Table 4.1 cont..

S. No.	Name of the descriptor	Descriptor state	No. of accessions	Genotypes	Frequency (%)
9	Leaf: Size	Small	4	RT-54, G.Til-4, RT-103, TKG-306	9.30
		Medium	32	Pratap, TKG-21, RT-125, RT-390, Swetha til, TKG-22, JCS-1020, RT-46, JCS-2454, CUMS-17, JTS-8, Chandana, VRI-2, RT-346, G.Til-2, Hima, RT-127, Gouri, YLM-11, G.Til-1, G.Til-3, Madhavi, G.Til-5, G.Til-6, G.Til-10, Nirmala, Rajeswari, TKG-55, TKG-308, E-8,JCS-3603, TMV 4	74.41
		Large	7	RT-351, YLM-17, YLM-66, YLM-146, TMV-7, VRI-4, Paiyur-1	16.27
10	Leaf: Serration of margin	Weak	3	RT-54, RT-46, Nirmala	6.97
		Strong	40	Pratap, TKG-21, RT-125,RT-390, Swetha til, TKG-22, JCS-1020, JCS-2454, CUMS-17, JTS-8, RT-351, Chandana, VRI-2, RT-346, G.Til-2, Hima, RT-127, Gouri, YLM-11, YLM-17, G.Til-1, G.Til-3, YLM-66, YLM-146, Madhavi, G.Til-4, G.Til-5, G.Til-6, G.Til-10, RT-103, Rajeswari, TKG-55, TKG-306, TKG-308, E-8,JCS-3603, TMV 4,TMV-7,VRI-4, Paiyur-1	93.02
11	Capsule: Hairiness	Absent	1	G.Til-10	2.32
		Sparse	34	TKG-21, RT-125, RT-390, TKG-22, JCS-1020, JCS-2454, CUMS-17, RT-351, VRI-2, RT-346, Hima, RT-127, Gouri, YLM-11, YLM-17, G.TIL-1, G.TIL-3, YLM-66, YLM-146, Madhavi, G.TIL-4, G.TIL-5, G.TIL-6, RT-103, Rajeswari, TKG-55, TKG-306, TKG-308, E-8, JCS-3603, TMV 4, TMV-7, VRI-4, Paiyur-1	79.06
		Dense	8	Pratap, RT-54, RT-46, JTS-8, Chandana, G.TIL-2, Nirmala, Swetha til	18.60
12	Capsule: Locule number/capsule	Four	43	Pratap, TKG-21, RT-125,RT-390, Swetha til, TKG-22, JCS-1020, RT-54, RT-46, JCS-2454, CUMS-17, JTS-8, RT-351, Chandana, VRI-2, RT-346, G.Til-2, Hima, RT-127, Gouri, YLM-11, YLM-17, G.Til-1, G.Til-3, YLM-66, YLM-146, Madhavi, G.Til-4, G.Til-5, G.Til-6, G.Til-10, RT-103, Nirmala, Rajeswari, TKG-55, TKG-306, TKG-308, E-8,JCS-3603, TMV-4,TMV-7,VRI-4, Paiyur-1	100
		Six	0	--	

Cont..

Table 4.1 cont..

S. No.	Name of the descriptor	Descriptor state	No. of accessions	Genotypes	Frequency (%)
13	Capsule: Shape	Tapered	0	--	-
		Narrow oblong	12	Pratap, G.Til-2, Hima, YLM-11, YLM-17, G.Til-1, YLM-146, G.Til-5, G.Til-10, RT-103, TMV 4, Paiyur-1	27.90
		Broad oblong	26	RT-54, JCS-2454, CUMS-17, JTS-8, RT-351, Chandana, RT-125, RT-346, RT-390, RT-127, Gouri, G.Til-3, YLM-66, Madhavi, G.Til-4, G.Til-6, Nirmala, Swetha til, Rajeswari, TKG-22, TKG-55, TKG-306, JCS-1020, JCS-3603, TMV-7, VRI-4	60.46
		Square	5	TKG-21, RT-46, VRI-2, TKG-308, E-8	11.62
14	Capsule: Number/leaf axil	One	37	Pratap, TKG-21, RT-125, RT-390, Swetha til, TKG-22, RT-54, RT-46, JCS-2454, CUMS-17, JTS-8, RT-351, Chandana, VRI-2, RT-346, Hima, RT-127, Gouri, YLM-11, YLM-17, YLM-66, YLM-146, Madhavi, G.Til-10, RT-103, Nirmala, Rajeswari, TKG-55, TKG-306, TKG-308, E-8,JCS-3603, TMV 4,TMV-7,VRI-4, Paiyur-1	86.04
		More than one	6	G.Til-1, G.Til-2, G.Til-4, G.Til-5, G.Til-6, JCS-1020	13.95
15	Capsule: Arrangement	Alternate	26	RT-125, RT-390, Swetha til, RT-54, CUMS-17, RT-351, Chandana, VRI-2, RT-346, Hima, RT-127, Gouri, YLM-11, YLM-17, G.Til-3, YLM-66, YLM-146, Madhavi, RT-103, Rajeswari, TKG-55, TKG-306, E-8,JCS-3603, TMV 4,TMV-7	60.46
		Opposite	11	Pratap, TKG 21, RT-46, JCS-2454, JTS-8, G.Til-10, Nirmala, TKG-22, TKG-308, VRI-4, Paiyur-1	25.58
		Cluster	6	G.Til-1, G.Til-2, G.Til-4, G.Til-5, G.Til-6, JCS-1020	13.95
16	Capsule: Length	Short (<1.5)	0	--	-
		Medium (1.5-2.5)	1	TMV-4	2.32
		Long (>2.5)	42	Pratap, TKG-21, RT-125,RT-390, Swetha til, TKG-22, JCS-1020, RT-54, RT-46, JCS-2454, CUMS-17, JTS-8, RT-351, Chandana, VRI-2, RT-346, G.Til-2, Hima, RT-127, Gouri, YLM-11, YLM-17, G.Til-1, G.Til-3, YLM-66, YLM-146, Madhavi, G.Til-4, G.Til-5, G.Til-6, G.Til-10, RT-103, Nirmala, Rajeswari, TKG-55, TKG-306, TKG-308, E-8,JCS-3603, TMV-7,VRI-4, Paiyur-1	97.67

Cont..

Table 4.1 cont..

S. No.	Name of the descriptor	Descriptor state	No. of accessions	Genotypes	Frequency (%)
17	Maturity: Days to maturity	Early (<75)	0	--	-
		Medium (76-85)	30	TKG-21, RT-125, RT-390, Swetha til, TKG 22, JCS 1020, RT-54, RT-46, JCS-2454, CUMS-17, JTS-8, RT-351, Chandana, RT-346, Hima, RT-127, Gouri, YLM-11, YLM-17, YLM-66, YLM-146, G.Til-4, G.Til-10, RT-103, TKG-55, TKG-306, TKG-308, JCS-3603, TMV-7,VRI-4	69.76
		Late (86-95)	13	Pratap, VRI-2, G.Til-2, G.Til-1, G.Til-3, Madhavi, G.Til-5, G.Til-6, Nirmala, Rajeswari, E-8, TMV 4, Paiyur-1	30.23
		Very late (>95)	0	--	-
18	Seed: Coat colour	White	27	Pratap, TKG-21, RT-46, JCS-2454, JTS-8, RT-351, RT-125, RT-346, RT-390, G.Til-2, Hima, RT-127, G.Til-1, G.Til-3, G.Til-4, G.Til-5, G.Til-6, RT-103, Swetha til, Rajeswari, TKG-22, TKG-55, TKG-306, TKG-308, E-8, JCS-1020, JCS-3603	62.79
		Grey	0	--	-
		Light brown	4	RT-54, CUMS-17, Chandana, Nirmala	9.30
		Dark brown	10	VRI-2, Gouri, YLM-11, YLM-17, YLM-66, YLM-146, Madhavi, TMV 4, TMV-7, VRI-4	23.25
		Black	2	G.Til-10, Paiyur-1	4.65
19	Seed: 1000 seeds weight(g)	Low (<2.5)	0	--	-
		Medium (2.5-3.0)	21	Pratap, TKG-21, JCS-2454, CUMS-17, RT-351, Chandana, RT-125, RT-346, RT-390, RT-127, Gouri, YLM-11, YLM-17, G.Til-1, YLM-146, RT-103, Nirmala, Rajeswari, TKG-22, E-8, TMV-4	48.83
		High (3.1-3.5)	22	RT-54, RT-46, JTS-8, VRI-2, G.Til-2, Hima, G.Til-3, YLM-66, Madhavi, G.Til-4, G.Til-5, G.Til-6, G.Til-10, Swetha til, TKG-55, TKG-306, TKG-308, JCS-1020, JCS-3603, TMV-7, VRI-4, Paiyur-1	51.16
		Very high (>3.5)	0	--	-
20	Seed oil: content (%)	Low (<45)	18	Pratap, RT-54, CUMS-17, RT-351, Chandana, RT-125, RT-346, RT-390, YLM-17, YLM-66, G.Til-5, G.Til-6, G.Til-10, RT-103, Nirmala, TKG-308, VRI-4, Paiyur-1	41.86
		Medium (45-50)	25	TKG-21, RT-46, JCS-2454, JTS-8, VRI-2, G.Til-2, Hima, RT-127, Gouri, YLM-11, G.Til-1, G.Til-3, YLM-146, Madhavi, G.Til-4, Swetha til, Rajeswari, TKG-22, TKG-55, TKG-306, E-8, JCS-1020, JCS-3603, TMV-4, TMV-7	58.13
		High (>50)	0	--	-

4.1.1.5 Plant: Branching

In case of branching, 23 genotypes (53.48 %) were classified as profuse branching (>4), 19 genotypes (44.18 %) as medium branching (2.1-4.0) and the remaining one genotype (2.32 %) as few (1-2). Profuse branching was predominant over medium and few branching, indicating their usefulness in plant breeding programmes as this is considered as one of the important yield contributing trait. The results found were in conformity with the findings of Valarmathi *et al.* (2003) and Pavani *et al.* (2020).

4.1.1.6 Plant: Branching pattern

Plant branching pattern was another distinguishing feature for varietal characterization. For this trait, among the genotypes tested, 23 genotypes (53.48 %) showed top branching pattern, while the rest of the 20 genotypes (46.51 %) displayed basal branching pattern. Top branching was predominant over basal branching. The results were in line with Sarita *et al.* (2013), Singh *et al.* (2017), Palakshappa *et al.* (2020) Pavani *et al.* (2020) and Vanishree *et al.* (2022). The genotypes Hima, Rajeswari, Pratap, YLM-66 and G.Til-4 recorded top branching pattern.

4.1.1.7 Stem: Hairiness

Hairiness is the significant character for natural defense mechanism for biotic and abiotic factors. So this character may be recognized as ideal plant type. Among 43 genotypes, 38 genotypes (88.37 %) exhibited sparse hairiness, four genotypes (9.30 %) exhibited absence of hair and one genotype (2.32 %) exhibited dense hairiness. Furat and Uzun (2010), Fray *et al.* (2015), Ozcinar and Sogut (2017), Singh *et al.* (2017), Palakshappa *et al.* (2020), Pavani *et al.* (2020) and Vanishree *et al.* (2022) also exploited this trait during their studies on characterization of sesame genotypes. The genotype RT-54 recorded dense stem hairiness.

4.1.1.8 Leaf: Lobes

Twenty nine genotypes (67.44 %) exhibited deeply lobed leaves whereas the other 14 genotypes (32.55 %) revealed slightly lobed leaves. Singh *et al.* (2017), Palakshappa *et al.* (2020), Pavani *et al.* (2020) and Vanishree *et al.* (2022) also used this trait as one of the criteria for characterization of genotypes in sesame. This trait is having direct effect on the photosynthesis and provides selective advantage in the use of irradiance to the function of petiole as vertical spacer in conditioning the competition to light and also plays an important role in increasing the biomass of the plant for easy characterization of genotypes Few among genotypes CUMS-17, JTS-8, Chandana, VRI-2, RT-346, RT-390 Pratap, Hima, YLM-66 and Nirmala recorded deeply lobed leaves.

4.1.1.9 Leaf: Size

For leaf size, 32 genotypes (74.41 %) exhibited medium leaf size, seven genotypes (16.27 %) exhibited large leaf size whereas the remaining four genotypes (9.30 %) showed small leaf size. Medium leaf size was predominant over small and large leaf size. The results are in consonance with Mathur *et al.* (2022). The genotypes Paiyur-1, YLM-66, YLM-146, TMV-7 and RT-251 exhibited large leaf size.

4.1.1.10 Leaf: Serration of margin

For the trait leaf serration, 40 genotypes (93.02 %) showed strong leaf serration whereas the rest of three genotypes (6.97 %) were with weak leaf serration indicates strong leaf serration is predominant over weak leaf serration.

4.1.1.11 Capsule: Hairiness

Out of 43 genotypes, 34 genotypes (79.06 %) possessed sparse capsule hair, eight genotypes (18.60 %) exhibited dense hairiness and one genotype (2.32 %) expressed absence of hair on capsule. Sparse hairiness were predominant and hairiness is considered to be useful for the pest and abitotic

stress resistance/tolerance. These results are akin with the findings of Ercan *et al.* (2002), Valarmathi *et al.* (2003), Suhasini *et al.* (2006), Parameshwarappa *et al.* (2008), Morris *et al.* (2009), Furat and Uzun (2010), Frary *et al.* (2015), Singh *et al.* (2017), Palakshappa *et al.* (2020), Pavani *et al.* (2020), Mathur *et al.* (2022). Vanishree *et al.* (2022). The genotypes Pratap, Nirmala, RT-54, Chandana and JTS-8 exhibited dense hairiness on capsule.

4.1.1.12 Capsule: Locule number per capsule

All the 43 genotypes of sesame exhibited four locule numbers per capsule (100.00 %) and therefore the trait could not distinguish the varieties from each other indicating that this trait may not be useful for discrimination of the varieties. Similar findings has been reported by Singh *et al.* (2017), Palakshappa *et al.* (2020), Pavani *et al.* (2020), Savaliya (2021) and Vanishree *et al.* (2022)

4.1.1.13 Capsule: Shape

Broad oblong capsule shape was observed in 26 genotypes (60.46 %) whereas, 12 genotypes (27.90 %) recorded narrow oblong capsule shape and five genotypes (11.62 %) appeared square capsule shape. The results found were in conformity with the findings of Singh *et al.* (2017), Ukani (2018), Mawcha *et al.* (2020), Palakshappa *et al.* (2020), Pavani *et al.* (2020) and Vanishree *et al.* (2022).

4.1.1.14 Capsule: Number per leaf axil

Out of 43 genotypes, 37 genotypes (86.04 %) exhibited one capsule per leaf axil and six genotypes (13.95 %) noted more than one capsule per leaf axil. This result was in consonance with the findings of Singh *et al.* (2017), Ukani (2018), Palakshappa *et al.* (2020), Pavani *et al.* (2020), Savaliya (2021) and Vanishree *et al.* (2022). The genotypes JCS-1020, G.Til-1, G.Til-2, G.Til-3, G.Til-4, G.Til-5 recorded more than one capsule per leaf axil.

4.1.1.15 Capsule: Arrangement

For capsule arrangement, 26 genotypes (60.46 %) exhibited alternate capsule arrangement, 11 genotypes (25.58 %) showed opposite capsule arrangement and six genotypes (13.95 %) revealed cluster capsule

arrangement. Alternate capsule arrangement was predominant over opposite and cluster arrangement. Similar results was obtained by Singh *et al.* (2017), Palakshappa *et al.* (2020), Pavani *et al.* (2020), Mathur *et al.* (2022) and Vanishree *et al.* (2022). The genotypes JCS-1020, G.Til-1, G.Til-2, G.Til-3, G.Til-4 and G.Til-5 recorded cluster capsule arrangement.

4.1.1.16 Capsule: Length (cm)

Forty two genotypes (97.67 %) showed long capsule length (>2.5 cm), whereas only one (2.32 %) genotype showed medium capsule length (1.5-2.5 cm). From the results it was clear that genotypes having long capsule length were predominant. The trait, capsule length, is considered to be an important yield contributing character and also used as a parameter for characterization of sesame genotypes. Similar classification was reported by Mahesh (2014), Azeez *et al.* (2016), Singh *et al.* (2017), Ukani (2018), Palakshappa *et al.* (2020), Pavani *et al.* (2020) and Vanishree *et al.* (2022).

4.1.1.17 Maturity: Days to maturity

Out of the 43 genotypes, 30 genotypes (69.76 %) showed medium days to maturity (76-85 days), while the remaining 13 genotypes (30.23 %) were late in maturity (86-95days). The result was in accordance with Singh *et al.* (2017), Palakshappa *et al.* (2020), Pavani *et al.* (2020) and Vanishree *et al.* (2022).

4.1.1.18 Seed: Coat colour

A wide range of variation is observed for seed coat colour ranging from white to black through all intermediate colours against reported white, black and brown. Twenty-seven genotypes (62.79 %) showed white seed colour, 10 genotypes (23.25 %) expressed dark brown seed colour, four genotypes (9.30 %) exhibited light brown (beige) seed colour and two genotypes (4.65 %) recorded black seed colour. Similar results were obtained by Suhasini *et al.* (2006), Azeez *et al.* (2016), Singh *et al.* (2017), Ukani (2018), Palakshappa *et al.* (2020), Pavani *et al.* (2020) and Vanishree *et al.* (2022).

4.1.1.19 Seed: Thousand seed weight (g)

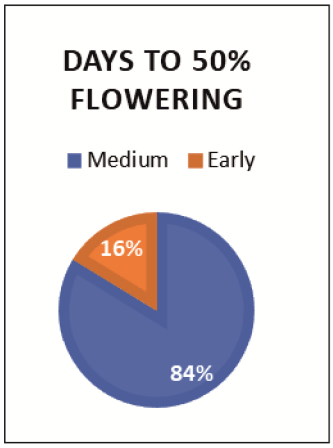
In case of 1000-seed weight, 22 genotypes (51.16 %) and 21 genotypes (48.83%) recorded high 1000-seed weight (3.1-3.5g) and medium (2.5-3.0g) 1000-seed weight respectively. High 1000-seed weight was predominant over low, medium and very high weights. This result was in agreement with the findings of Singh *et al.* (2017), Palakshappa *et al.* (2020), Pavani *et al.* (2020) and Vanishree *et al.* (2022).

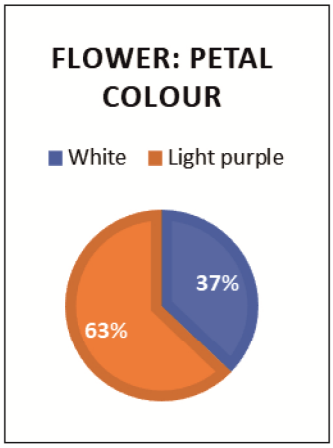
4.1.1.20 Seed oil: Content (%)

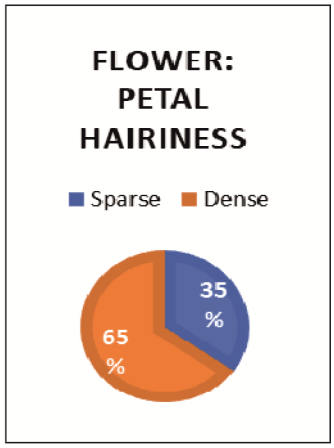
Out of 43 genotypes, twenty five genotypes (58.13 %) exhibited medium oil content (45-50 %) whereas 18 genotypes (41.86 %) deciphered low oil content (>45 %). Singh *et al.* (2017), Palakshappa *et al.* (2020), Pavani *et al.* (2020) and Vanishree *et al.* (2022) also exploited this trait during their studies on characterization of sesame genotypes.

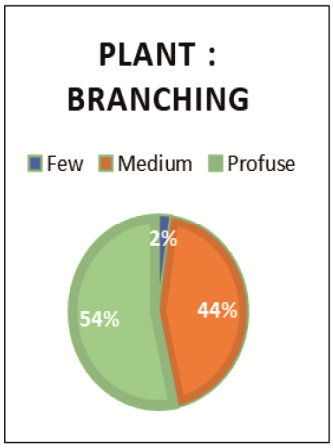
Based on characterisation of the 43 genotypes of sesame for 20 morphological traits majority of sesame accessions were found to be possessing medium duration flowering (83.72 %), light purple petal colour (62.79 %), dense petal hairiness (65.11 %), medium plant height (100.00 %), profuse branching (53.48 %), top branching (53.48 %), sparse stem hairiness (88.37 %), deeply lobed (67.44 %), medium leaf size (74.41 %), strong leaf serration (93.02 %), sparse capsule hairiness (79.06 %), four locule numbers per capsule (100.00 %), broad oblong capsule shape (60.46 %), one capsule per leaf axil (86.04 %), alternate capsule arrangement (60.46 %), long capsule length (97.67 %), medium days to maturity (69.76 %), white seed colour (62.79 %), high 1000-seed weight (51.16 %) and medium oil content (58.13 %). Based on the above results it could be divulged that all the traits were quite informative with respect to the trait expression cum characterization.

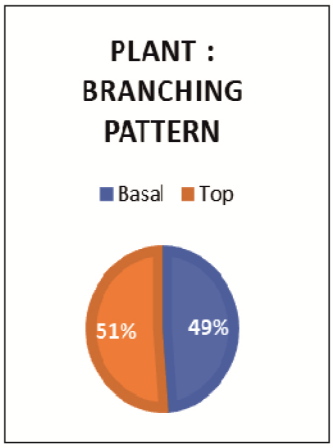
In conclusion, present study revealed the distinctness among the sesame accessions for morphological characteristics studied indicating the morphological variations among lines is due to variation in the genetic makeup. The identified DUS traits will serve as markers in selection process for genotype identification.

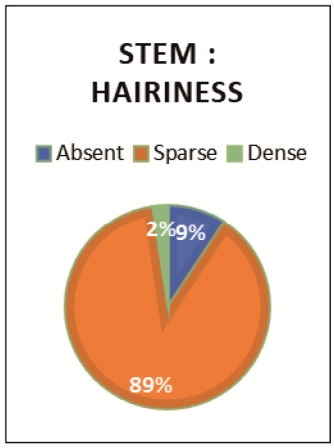

DAYS TO 50% FLOWERING
Medium
Early
16%
84%

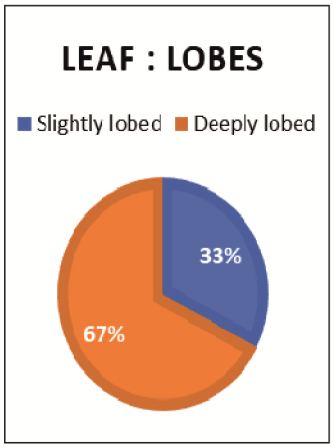

FLOWER: PETAL COLOUR
White
Light purple
37%
63%

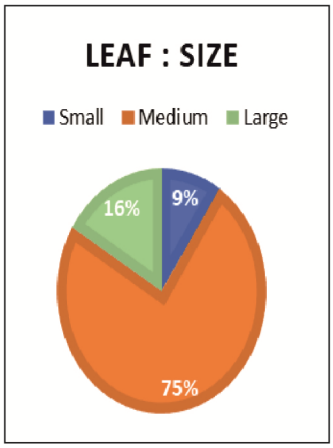

FLOWER: PETAL HAIRINESS
Sparse
Dense
35 %
65 %

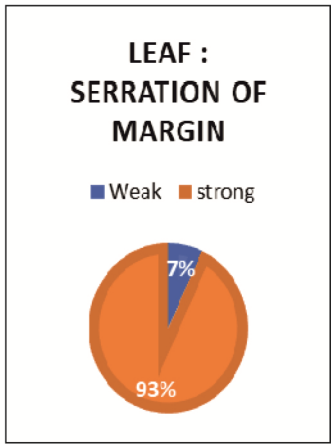

PLANT : BRANCHING
Few
Medium
Profuse
2%
44%
54%


PLANT : BRANCHING PATTERN
Basal
Top
51%
49%


STEM : HAIRINESS
Absent
Sparse
Dense
2%
9%
89%


LEAF : LOBES
Slightly lobed
Deeply lobed
33%
67%


LEAF : SIZE
Small
Medium
Large
16%
9%
75%


LEAF : SERRATION OF MARGIN
Weak
strong
7%
93%

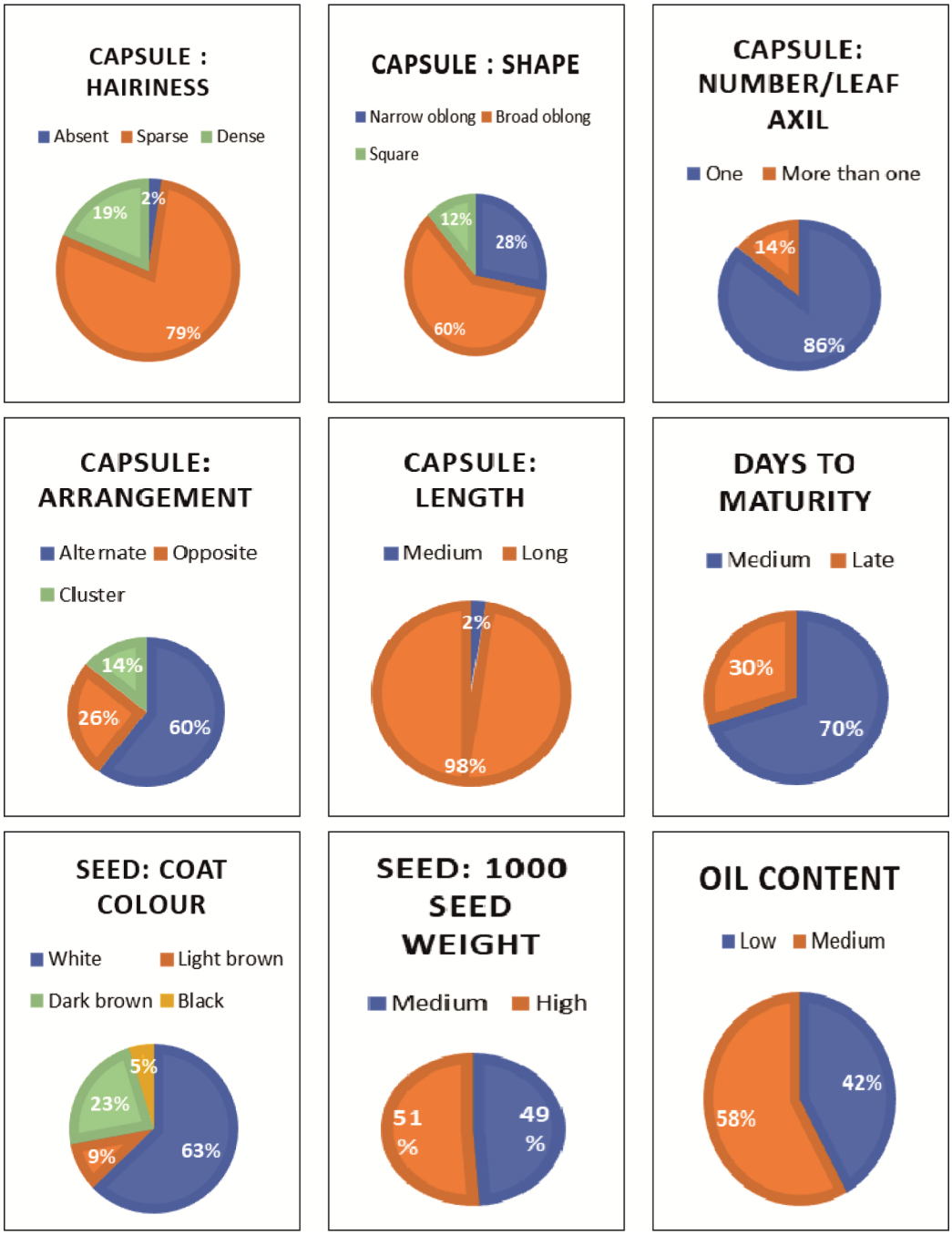


Fig. 4.1. Pie diagram depicting variability for DUS traits among 43 genotypes of sesame

White petal colour
Dense petal hairiness
(Pratap)

White petal colour
Sparse petal hairiness
(JCS-2454)

Light Purple petal colour
Dense petal hairiness
(G.Til-4)

Light Purple petal colour
Sparse petal hairiness
(CUMS-17)

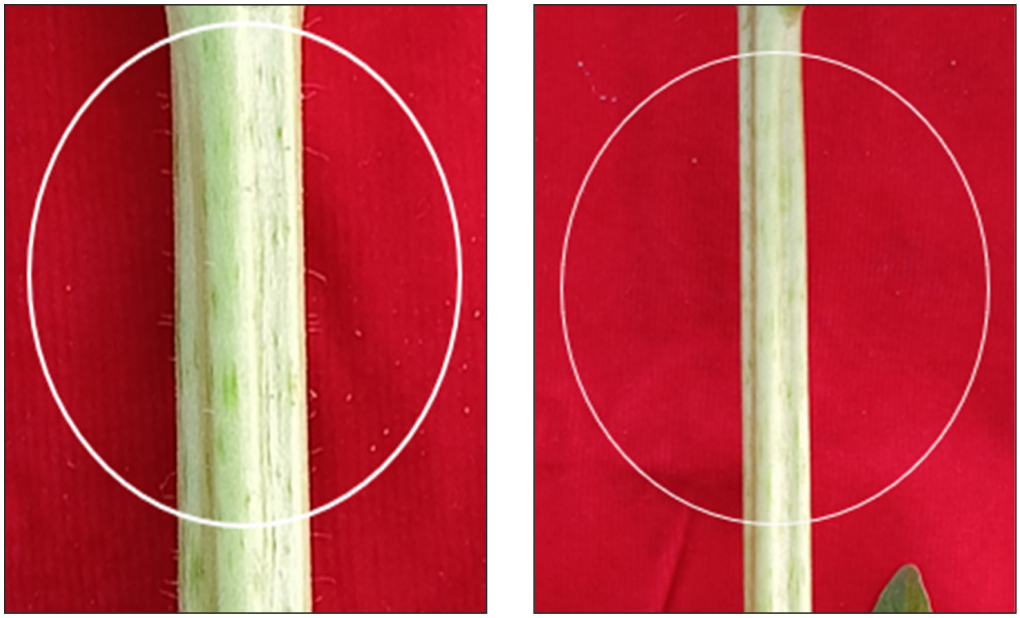
Plate 2: Representative photographs of flower petal colour and hairiness

Profuse branching (CUMS-17) | **Medium branching (Rajeswari)** | **Few branching (JCS-1020)**

Plate 3: Plant: Branching

Top branching (Pratap) | **Basal branching (TKG-21)**

Plate 4: Plant: Branching pattern



Sparse hairiness (YLM-66) | **Absence of hair (TMV-4)**

Plate 5: Stem: Hairiness

Slightly lobed (TKG-22) | **Deeply lobed (Pratap)**

Plate 6: Leaf: Lobes

Small (RT-54) | **Medium (Chandana)** | **Large (YLM-17)**

Plate 7: Leaf: Size

Weak serration (Nirmala) | **Strong serration (Rajeswari)**

Plate 8: Leaf: Serration of margin

Dense hair
(Nirmala)

Sparse hair
(Paiyur-1)

Absence of hair
(G.Til-10)

Plate 9: Capsule: Hairiness

Narrow oblong (YLM-17) | **Broad oblong (Nirmala)** | **Square (VRI-2)**

Plate 10: Capsule: Shape

Single capsule (Gouri) | **Multicapsular (G.Til-1)**

Plate 11: Capsule: Number per leaf axil

Alternate (Gouri) **Opposite (TKG-308)** **Cluster (G.Til-1)**

Plate 12: Capsule: Arrangement

Plate 13: Seed: Coat colour

4.2 ANALYSIS OF VARIANCE

Analysis of variance for 10 quantitative characters *viz.,* days to 50% flowering, days to maturity, plant height (cm), number of primary branches per plant, number of capsules per plant, capsule length (cm), number of seeds per capsule, 1000-seed weight (g), seed yield per plant (g), oil content (%) were presented in the Table 4.2. and revealed that mean sum of squares were highly significant for all the characters studied, suggesting the presence of substantial magnitude of genetic variability among the genotypes.

Table 4.2 Analysis of Variance for yield and yield attributing characters in 43 sesame genotypes

S. No.	Characters	Mean sum of squares		
		Replication (df=2)	Genotype (df=42)	Error (df=84)
1	Days to 50 % flowering	10.90	31.87**	3.62
2	Days to maturity	2.88	29.24**	0.93
3	Plant height (cm)	84.67	156.56**	30.38
4	Number of primary branches per plant	0.31	2.03**	0.15
5	Number of capsules per plant	4.86	33.54**	1.60
6	Capsule length (cm)	0.02	0.12**	0.01
7	Number of seeds per capsule	23.11	97.23**	16.42
8	1000-seed weight (g)	0.10	0.11**	0.05
9	Oil content (%)	4.86	33.54**	1.61
10	Seed yield per plant (g)	0.18	44.16**	7.04

** Significance at 1% level

4.3 MEAN PERFORMANCE

Mean performance is a simple measure used in plant breeding to assess phenotypic variability and it serves as a basis for eliminating undesirable genotypes. The mean performance of 43 sesame genotypes for all the traits studied are presented character wise in the Table 4.3.

Table 4.3 Mean performance of 43 sesame genotypes for ten yield and its attributing characters

S.No	Genotype	DFF	DM	PH	NPB	NCP	CL	NSC	TSW	OC	SYP
1	Pratap	32.00	90.66	92.73	2.73	74.07	3.14	72.80	2.87	43.54	10.47
2	TKG-21	34.33	85.00	99.89	4.00	60.47	3.04	73.33	2.90	46.01	9.97
3	RT-54	39.00	83.33	92.83	4.00	78.33	2.94	67.20	3.07	32.31	13.77
4	RT-46	37.00	80.67	93.07	4.33	85.87	3.04	63.93	3.27	45.52	19.70
5	JCS-2454	42.33	85.00	100.67	3.47	68.27	3.27	68.67	2.87	46.38	10.37
6	CUMS-17	42.00	82.00	102.67	4.07	80.60	2.94	84.00	2.93	37.90	12.10
7	JTS-8	38.33	82.00	100.03	4.27	75.73	3.22	73.00	3.13	46.31	10.23
8	RT-351	36.33	82.33	98.40	2.73	60.33	3.12	75.20	2.93	42.90	8.97
9	Chandana	41.67	81.67	98.93	5.23	76.27	2.99	72.80	2.83	37.15	12.57
10	VRI-2	41.00	89.33	107.60	4.73	76.80	2.69	65.60	3.23	46.68	13.80
11	RT-125	34.00	83.33	91.77	4.40	51.06	3.18	76.67	2.90	43.75	8.57
12	RT-346	40.33	84.67	105.57	3.67	56.10	2.95	70.40	2.87	42.58	9.40
13	RT-390	35.33	81.67	102.33	3.67	87.13	3.11	69.33	2.97	43.49	11.10
14	G.Til-2	41.33	89.00	88.20	2.73	75.07	2.93	73.20	3.40	46.99	16.00
15	Hima	42.00	78.33	106.00	5.07	93.80	2.96	67.20	3.25	45.12	21.70
16	RT-127	37.33	84.33	105.67	3.60	61.67	3.18	73.73	2.93	45.23	9.33
17	Gouri	42.67	84.67	95.40	4.67	62.67	2.84	71.93	3.03	45.97	14.10
18	YLM-11	43.00	79.00	98.73	5.33	80.80	2.93	68.27	2.90	46.61	9.70
19	YLM-17	42.00	81.33	95.60	4.63	57.93	2.92	65.87	3.00	43.76	8.00
20	G.Til-1	40.67	88.33	83.80	2.73	74.20	2.78	71.73	2.93	48.04	18.50
21	G.Til-3	41.67	89.00	97.07	3.33	78.47	3.51	75.60	3.23	45.76	13.77
22	YLM-66	42.67	81.00	101.77	4.40	85.93	2.73	68.27	3.33	44.22	18.13
23	YLM-146	42.33	80.00	109.20	4.53	78.07	2.97	62.40	2.97	45.86	18.63
24	Madhavi	42.00	86.00	98.07	5.03	62.13	2.90	81.33	3.13	46.40	11.40
25	G.Til-4	40.33	84.33	86.67	3.13	62.40	2.71	65.67	3.53	47.88	10.53

Cont..

Table 4.3 cont..

S.No	Genotype	DFF	DM	PH	NPB	NCP	CL	NSC	TSW	OC	SYP
26	G.Til-5	41.33	86.67	82.10	4.03	84.73	3.03	66.47	3.27	44.77	16.37
27	G.Til-6	36.67	86.33	104.27	2.93	56.67	3.17	65.07	3.17	43.05	12.00
28	G.Til-10	41.00	82.33	98.37	4.33	87.20	2.78	64.40	3.10	42.27	13.87
29	RT-103	36.67	80.67	93.78	4.47	90.27	3.11	74.13	3.03	41.73	15.20
30	Nirmala	42.00	86.00	98.07	4.40	87.47	2.67	70.53	2.87	35.49	18.23
31	Swetha Til	32.67	83.67	99.00	4.60	66.93	3.25	69.87	3.13	47.06	8.37
32	Rajeswari	36.33	86.00	91.27	3.17	71.40	3.27	75.93	2.97	48.80	12.10
33	TKG-22	32.33	85.00	87.50	4.13	60.07	3.21	90.60	2.93	46.73	9.83
34	TKG-55	40.00	84.67	97.80	4.27	53.20	3.34	67.07	3.37	45.09	8.97
35	TKG-306	38.33	83.33	90.37	4.00	70.27	2.94	64.40	3.33	45.98	11.30
36	TKG-308	41.67	84.33	84.37	4.00	86.27	2.95	71.20	3.43	43.23	19.70
37	E-8	40.67	89.33	101.80	3.73	82.60	3.05	72.40	2.93	47.79	17.23
38	JCS-1020	33.67	83.00	99.07	1.33	68.47	2.98	68.27	3.37	47.10	10.47
39	JCS-3603	41.00	85.00	103.20	4.33	82.13	2.84	65.33	3.33	45.84	16.40
40	TMV-4	41.00	90.00	92.13	4.00	88.60	2.50	66.40	2.97	45.21	15.83
41	TMV-7	42.33	84.00	111.93	4.33	73.20	2.69	65.87	3.30	45.50	13.97
42	VRI-4	42.00	81.67	110.20	4.67	85.00	2.90	67.07	3.20	42.49	18.77
43	Paiyur-1	36.67	90.00	104.20	5.40	89.37	3.20	77.73	3.23	44.27	19.73
Mean		**39.25**	**84.39**	**97.72**	**4.01**	**74.13**	**2.99**	**70.71**	**3.10**	**44.38**	**13.46**
Min		32.00	78.33	82.10	1.33	51.06	2.50	62.40	2.83	32.31	8.00
Max		43.00	90.66	111.93	5.40	93.80	3.51	90.60	3.53	48.80	21.70
SE(m)		1.09	0.55	3.17	0.23	4.05	0.07	2.33	0.13	0.73	1.53
CD (5%)		3.08	1.57	8.93	0.64	11.41	0.22	6.58	0.37	2.05	4.31
CV %		4.84	1.14	5.63	9.95	9.48	4.52	5.73	7.40	2.85	19.71

DFF : Days to 50% flowering
DM : Days to maturity
PH : Plant height (cm)
NPB : Number of primary branches per plant
NCP : Number of capsules per plant
CL : Capsule length (cm)
NSC : Number of seeds per capsule
TSW : 1000 seed weight (g)
OC : oil content (%)
SYP : Seed yield per plant (g)

4.3.1 Days to 50 % Flowering

Days to 50 % flowering varied from 32.00 days to 43.00 days with general mean of 39.25 days. Among all genotypes, Pratap (32 days) was earliest to flower whereas YLM-11 (43 days) was late in flowering. Out of 43 genotypes evaluated, seventeen genotypes were earlier in flowering and twenty-six were late in flowering when compared with general mean (39.25 days). Pratap, TKG-22, Swetha til, JCS-1020, RT-125 were earliest to flower indicating that these genotypes could be exploited as donors for evolving short duration varieties.

4.3.2 Days to Maturity

The number of days to maturity ranged from 78.33 to 90.66 days with a mean maturity of 84.39 days. The genotype Hima came to early in maturity (78.33 days), while Pratap was found to be late in maturity (90.66 days). Twenty three genotypes were early and 20 genotypes were late in maturity when compared with the mean maturity of the genotypes (84.39 days). Hima, YLM-11, YLM-146, RT-46 and RT-103 were earliest to mature indicating these genotypes were used to develop sesame varieties with different maturity groups to fit into different farming situations under different agro-climatic zones.

4.3.3 Plant Height at Maturity (cm)

The mean value for plant height ranged from 82.10 cm to 111.93 cm with general mean of 97.72 cm. G.Til-5 (82.10 cm) was found to be shortest whereas TMV-7 (111.93cm) was the tallest among all the genotypes. Twenty six genotypes were taller in height compared to general mean height (97.72cm). The genotype TMV-7 was tallest followed by VRI-4, YLM-146, VRI-2 and Hima. Hence, these genotypes could be suitable to develop tall genotypes, as plant height is directly proportional to number of capsules, which ultimately increases yield.

4.3.4 Number of primary branches per plant

The highest number of primary branches per plant was recorded by the genotype Paiyur-1 (5.40), whereas the lowest value was registered by JCS-1020 (1.33). Twenty four genotypes showed more number of primary branches per plant than the general mean of the genotypes (4.01). Paiyur-1 followed by YLM-11, Chandana, Hima, Madhavi registered registered more number of primary branches per plant.

4.3.5 Number of capsules per plant

A range of 51.06 to 93.80 capsules per plant was observed with a general mean of 74.13. The maximum number of capsules per plant was recorded by the genotype Hima (93.80), whereas it was minimum in RT-125 (51.06). Twenty four genotypes showed more number of capsules per plant when compared to the general mean of this trait (74.13). Hima, RT-103, Paiyur-1, TMV-4 and Nirmala recorded more number of capsules per plant. Hence, these genotypes could be utilized in the hybridization programme to improve the seed yield.

4.3.6 Capsule length (cm)

Capsule length ranged from 2.50 cm to 3.51 cm with general mean of 2.99 cm. G.Til-3 (3.51cm) recorded high capsule length whereas TMV-4 (2.50 cm) recorded low capsule length. Twenty genotypes registered high capsule length than general mean of 2.99 cm. G.Til-3, TKG-55, Rajeswari, JCS-2454 and Swetha til recorded high capsule length.

4.3.7 Number of seeds per capsule

YLM-146 (62.40) recorded less number of seeds per capsule whereas TKG-22 (90.60) recorded more number of seeds per capsule compared to general mean 70.71. Nineteen genotypes showed more seeds per capsule than general mean (70.71). TKG-22, CUMS-17, Madhavi, Paiyur-1 and RT-125 recorded for number of seeds per capsule.

4.3.8 Thousand seed weight (g)

The genotype G.Til-4 recorded the maximum 1000-seed weight (3.53g), whereas, Chandana recorded lowest (2.83g) with a general mean of 3.10g. Twenty genotypes exceeded the general mean 1000-seed weight (3.10g). High 1000-seed weight was registered by the G.Til-4, TKG-308, G.Til-2, JCS-1020 and TKG-55 which could be included in the hybridization programme for improving the yield. Thousand seed weight is one of the important attribute among the yield components that directly correlates with the yield.

4.3.9 Oil content (%)

Oil content ranged from 32.31 % to 48.80 % with general mean of 44.38%. Rajeswari (48.80 %) recorded high oil content whereas RT-54 (32.31 %) recorded low oil content. Twenty-six genotypes surpassed general mean of 44.38 %. Rajeswari, G.Til-1, G.Til-4, E-8 and JCS-1020 could be given higher preference to improve the oil content.

4.3.10 Seed yield per plant (g)

The trait seed yield per plant ranged from 8.00g to 21.70g. The genotype, Hima (21.70g) recorded highest seed yield per plant whereas YLM-17 (8.00g) registered lowest seed yield per plant. Twenty one genotypes exceeded the mean value of seed yield (13.46g). Hima exhibited high seed yield followed by Paiyur-1, TKG-308, RT-46 and VRI-4. Hence, these genotypes had greater potential to utilize in future breeding programme aimed at high yield.

In any plant breeding programme for development of high yielding hybrids or varieties the basic need is the choice of parents with high mean values as they are expected to produce desirable segregants upon crossing (Gilbert, 1958).

From overall mean estimates, it could be observed that several genotypes showed high mean performance for more than one character. The

genotype Hima exhibited high *per se* performance for seed yield per plant along with days to maturity, plant height, number of primary branches per plant and number of capsules per plant. Similarly, Paiyur-1 displayed higher *per se* performance for four traits *viz.,* number of primary branches per plant, number of capsules per plant, number of seeds per capsule and seed yield per plant. Further, the genotype JCS-1020 showed high mean values for days to 50 % flowering,1000-seed weight and oil content. Hence, it could be suggested that selection of these genotypes in hybridization programme would be effective for improvement of seed yield along with high oil content.

By and large, based on *per se* performance, it could be concluded that, the following genotypes (Table 4.4) showed outstanding mean performance with respect to different characters and these genotypes could be exploited as potential donors in breeding programme aimed for improvement of the respective traits and in turn improvement of yield in sesame.

Table 4.4 List of promising genotypes identified for yield and yield attributing characters in sesame

S.No	Traits	Genotypes
1	Days to 50 % flowering	Pratap, TKG-22, Swetha til, JCS-1020, RT-125
2	Days to maturity	Hima, YLM-11, YLM-146, RT-46, RT-103
3	Plant height (cm)	TMV-7, VRI-4, YLM-146, VRI-2, Hima
4	Number of primary branches per plant	Paiyur-1, YLM-11, Chandana, Hima, Madhavi
5	Number of capsules per plant	Hima, RT-103, Paiyur-1, TMV-4, Nirmala
6	Capsule length (cm)	G.Til-3, TKG-55, Rajeswari, JCS-2454, Swetha til
7	Number of seeds per capsule	TKG-22, CUMS-17, Madhavi, Paiyur-1, RT-125
8	1000-seed weight (g)	G.Til -4, TKG-308, G.Til -2, JCS-1020, TKG-55
9	Oil content (%)	Rajeswari, G.Til -1, G.Til -4, E-8, JCS-1020
10	Seed yield per plant (g)	Hima, Paiyur-1, TKG-308, RT-46, VRI-4

4.4 GENETIC PARAMETERS

4.4.1 Variability

The success of any crop improvement depends on the existence of genetic variability for yield and its components in the base population. This is sine qua non to high yielding cultivar development. Selection will be effective when there is a significant amount of variability among the breeding materials. So, the assessment of genetic variability in the base population should have to be prior action in breeding program. Information on the relative magnitude of different sources of variation among different genotypes for several traits helps in measurement of their range of genetic diversity. The genetically diverse genotypes are likely to produce heterotic effect and superior segregants when incorporated in hybridization to hasten crop improvement program. The available variability can be measured using genotypic and phenotypic coefficient of variation which used to partition genetic and environmental variance.

The variability estimates *viz*., phenotypic and genotypic coefficient of variation, heritability in broad sense, genetic advance and genetic advance as per cent of mean computed for 10 yield and yield contributing traits in 43 genotypes of sesame are furnished in Table 4.5.

The perusal of estimates revealed that phenotypic coefficient of variation was slightly higher than the genotypic coefficient of variation (GCV) for all the traits. In the present study wide range of variation was observed for all the traits indicating the influence of environment on these traits. The character seed yield per plant (GCV: 26.12 %; PCV: 32.72 %) showed higher estimates of PCV and GCV indicating ample amount of variation among genotypes for this trait. Therefore, simple selection would be effective for further improvement of these character. These results are in accordance with Sumathi *et al.* (2011), Iqbal *et al.* (2016), Patil and Lokesha (2018), Kalaiyarasi *et al.* (2019) and Sirisha *et al.* (2020).

Table 4.5 Variability and genetic parameters of 43 sesame genotypes for yield and yield attributing characters

S. No.	Character	Mean	Range		Variance		Coefficient of Variation		Heritability (Broad sense) %	Genetic Advance (GA)	Genetic Advance as percent of mean (%)
			Min.	Max.	Genotypic	Phenotypic	Genotypic	Phenotypic			
1	Days to 50 % flowering	**39.25**	32.00	43.00	9.42	13.04	7.82	9.20	72.20	5.37	13.69
2	Days to maturity	**84.39**	78.33	90.67	9.43	10.37	3.64	3.82	90.90	6.03	7.15
3	Plant height (cm)	**97.72**	82.10	111.93	42.09	72.38	6.64	8.71	58.20	10.19	10.43
4	Number of primary branches per plant	**4.01**	1.33	5.40	0.63	0.79	19.71	22.08	79.70	1.46	36.24
5	Number of capsules per plant	**74.13**	51.06	93.80	119.17	168.62	14.72	17.51	70.70	18.90	25.05
6	Capsule length (cm)	**2.99**	2.50	3.51	0.04	0.06	6.37	7.81	66.50	0.32	10.71
7	Number of seeds per capsule	**70.71**	62.40	90.60	26.94	43.36	7.34	9.31	62.10	8.43	11.92
8	1000-seed weight (g)	**3.10**	2.83	3.53	0.02	0.07	4.48	8.65	26.90	0.15	4.79
9	Oil content (%)	**44.38**	32.31	48.80	10.65	12.25	7.35	7.89	86.80	6.26	14.11
10	Seed yield per plant (g)	**13.46**	8.00	21.70	12.37	19.42	26.12	32.72	63.70	5.78	42.94

Moderate estimates of GCV and PCV are observed for number of capsules per plant (GCV: 14.72 %; PCV: 17.51 %). Durga *et al.* (2014), Hika *et al.* (2015) and Umamaheswari *et al.* (2019) reported moderate GCV and PCV for number of capsules per plant. Moderate estimates of GCV and higher estimates of PCV are observed for number of primary branches per plant (GCV: 19.71 %; PCV: 22.08 %). This was in conformity with the findings of Teklu *et al.* (2014), Abate *et al.* (2015), Vamshi *et al.* (2021). This indicates the existence of comparatively moderate variability for these traits hence, phenotypic selection of such traits in advanced generations would be effective.

Lower estimates of GCV and PCV were observed for days to 50 % flowering (GCV: 7.82 %; PCV: 9.20 %), Oil content (GCV: 7.35 %; PCV: 7.89 %), number of seeds per capsule (GCV: 7.34 %; PCV: 9.31 %), plant height (GCV: 6.64 %; PCV: 8.71 %), capsule length (GCV: 6.37 %; PCV: 7.81 %), 1000-seed weight (GCV: 4.48 %; PCV: 8.65 %) and days to maturity (GCV: 3.64 %; PCV: 3.82 %) in the decreasing order of their magnitude indicating the low range of variation for these characters in the genotypes, thus offering little scope for further improvement of these characters through simple selection. Improvement in these characters can be brought about by hybridization to widen genetic base followed by selection in advanced generations. These were in accordance with the findings of Patil and Lokesha (2018), Mohanty *et al.* (2020), Singh *et al.* (2022) for days to 50 % flowering and days to maturity; Bhattacharjee *et al.* (2019) for number of seeds per capsule; Teklu *et al.* (2014), Tripathy *et al.* (2016), Pavani *et al.* (2020) for oil content; Tripathi *et al.* (2013), Hika *et al.* (2015) for plant height; Ukaan *et al.* (2012), Saxena and Bisen (2017), Patil and Lokesha (2018), Bhattacharjee *et al.* (2019), Pavani *et al.* (2020) for capsule length; Saxena and Bisen (2017) and Pavani *et al.* (2020) for 1000-seed weight .

However, the difference was narrow between PCV and GCV for the traits *viz*., days to maturity, oil content, plant height, days to 50 % flowering, capsule length and number of seeds per capsule indicating negligible

influence of environmental factors in the expression of characters and selection on the basis of phenotype independent of genotype could be effective for the improvement of these traits. In contrast, the difference was wide for seed yield per plant and 1000-seed weight suggesting that influence of environment plays a role in expression of these traits and warranted further improvement through selection. The results obtained in our study is in agreement with the findings from Khan *et al.* (2007), Haibru *et al.* (2018), Mohanty *et al.* (2020) for days to maturity; Pavani *et al.* (2020) for oil content; Khan *et al.* (2007), Sumathi *et al.* (2011), Umamaheswari *et al.* (2019), Sasipriya *et al.* (2022) for plant height; Mohanty *et al.* (2020), Pavani *et al.* (2020), Kant *et al.* (2021) for days to 50 % flowering; Haibru *et al.* (2018), Sasipriya *et al.* (2022) for capsule length and number of seeds per capsule; Khan *et al.* (2007), Iqbal *et al.* (2016), Pavani *et al.* (2020), Kumari *et al.* (2023) for seed yield per plant; Umamaheswari *et al.* (2019), Mohanty *et al.* (2020), Sasipriya *et al.* (2022) for 1000-seed weight.

4.4.2 Heritability ($h^2_{(b)}$)

The existence of genetic variability is essential for selection, which is the ultimate tool in plant breeding. However, the selection is challenging due to the masking effect of non-heritable variation. Therefore, partitioning of the total variation to understand the role of the heritable component is very important, which will help the breeder to formulate a sound breeding programme. The heritability estimate separates the environmental influence from the total variability and indicates the accuracy with which a genotype can be identified by its phenotypic performance, thus, making the selection more effective. As such the heritability in a broader perspective is the proportion of genotypic variability to the total variability. Its importance has been emphasized by Lush (1949) in animals and Johnson *et al.* (1955) in plants. In the present study, heritability in broad sense was estimated and presented in Table 4.5.

In the present study the characters *viz.*, days to maturity (90.90 %), oil content (86.80 %), number of primary branches per plant (79.70 %), days to 50 % flowering (72.20 %), number of capsules per plant (70.70 %), capsule length (66.50 %), seed yield per plant (63.70 %) and number of seeds per capsule (62.10 %) exhibited high heritability (broad sense) indicating that these characters were least influenced by environmental effect and selection may be effective as these remain stable under varied environmental conditions. This also suggesting that the phenotypes are the true representative of their genotypes for these traits and selection based on phenotypic value could be reliable. Therefore, for improvement of these traits the selection will be more effective in early generation on the basis of *per se* performance.

These results are in line with the findings of Saxena and Bisen (2017), Kiruthika *et al.* (2018) for oil content; Kumari *et al.* (2020), Sahu *et al.* (2022) for number of primary branches per plant; Sabiel *et al.* (2015), Divya *et al.* (2018), Mohanty *et al.* (2020) for days to 50% flowering; Patil and Lokesha (2018), Umamaheswari *et al.* (2019) for number of capsules per plant; Haibru *et al.* (2018) for capsule length; Mahmoud *et al.* (2015), Saxena and Bisen (2017), Sultana *et al.* (2019) for seed yield per plant; Durga *et al.* (2014), Imran *et al.* (2018), Singh *et al.* (2022) for number of seeds per capsule.

A moderate estimate of heritability was observed for plant height (58.20 %). This was akin with the findings of Tripathy *et al.* (2016) and Patel *et al.* (2022) who reported moderate heritability for plant height. 1000-seed weight registered lower heritability (26.90 %). Similar results reported by Menzir *et al.* (2012) and Singh *et al.* (2022).

4.4.3 Genetic advance (GA)

Heritability estimates in the broad sense alone is not a true indicator of effective selection for the trait since their scope is restricted by their interactions with the environment. Thus, selection of traits based on heritability and genetic advance as per cent of mean is of great importance to

the breeder for making criteria for improvement in a complex trait. The knowledge on heritability in conjunction with genetic advance is most useful in predicting the scope of genetic improvement through selection (Johnson *et al.*, 1955). Genetic advance is the improvement in the mean of selected families over the base population. It is the measure of genetic gain under selection.

In the present study, majority of the traits *viz.,* number of seeds per capsule (8.43), oil content (6.26), days to maturity (6.03), seed yield per plant (5.78), days to 50 % flowering (5.37), number of primary branches per plant (1.46), capsule length (0.32) and 1000-seed weight (0.15) registered low genetic advance, while number of capsules per plant (18.90) and plant height (10.19) recorded moderate genetic advance.

4.4.4 Genetic advance as per cent of mean (GAM)

Estimates of heritability and genetic advance were interpreted together in order to predict the genetic gain under selection. Heritability and genetic advance when considered together would be more reliable and useful in predicting the resultant effects of selection (Johnson *et al.*, 1955). Rapid progress in selection can be achieved when high heritability is accompanied with high genetic advance, which forms the most reliable index of selection (Burton, 1952).

The characters *viz.,* seed yield per plant (42.94), number of primary branches per plant (36.24) and number of capsules per plant (25.05) exhibited high genetic advance as percent mean, while moderate genetic advance as percent mean was noted for the traits *viz.,* oil content (14.11), days to 50 % flowering (13.69), number of seeds per capsule (11.92), capsule length (10.71) and plant height (10.43). In contrast, low genetic advance as percent mean was observed by days to maturity (7.15) and 1000-seed weight (4.79).

In the present study, high heritability coupled with high genetic advance as percent mean were exhibited by number of primary branches per plant (h^2_b = 79.70%, GAM =36.24), number of capsules per plant (h^2_b =

70.70%, GAM = 25.05 %) and seed yield per plant (h^2_b = 63.70%, GAM = 42.94%) indicating the preponderance of additive gene action and high selective value thus, selection pressure could profitably be applied on these characters for their improvement. These results corroborates with the findings of Kalaiyarasi *et al.* (2019), Roy *et al.* (2022) for number of primary branches per plant; Bharathi *et al.* (2014), Bindu *et al.* (2014), Soundarya *et al.* (2016), Kalaiyarasi *et al.* (2019), Kehie *et al.* (2020) for number of capsules per plant; Bharathi *et al.* (2014), Soundarya *et al.* (2016), Saxena and Bisen (2017), Manjeet *et al.* (2020) for seed yield per plant.

High heritability coupled with moderate genetic advance as percent mean were exhibited by oil content (h^2_b = 86.80 %, GAM = 14.11 %), days to 50 % flowering (h^2_b = 72.20 %, GAM = 13.69 %), capsule length (h^2_b = 66.50 %, GAM = 10.71 %) and number of seeds per capsule (h^2_b = 62.10 %, GAM = 11.92 %) suggesting that these characters were likely to be controlled by both additive gene and non-additive gene action. Hence, direct and simple selection could be exercised due to fixable additive gene effects. These results were consonance with Kumari *et al.* (2023) for oil content; Tripathi *et al.* (2013), Bharathi *et al.* (2014), Singh *et al.* (2018), Vamshi *et al.* (2021) for days to 50% flowering; Haibru *et al.* (2018) for capsule length; Bhuiyan *et al.* (2019), Patel *et al.* (2022) for number of seeds per capsule.

High heritability coupled with low genetic advance as percent mean was exhibited by days to maturity (h^2_b = 90.90%, GAM = 7.15 %) indicating that this trait was governed by non-additive gene action. Hence selection could be postponed for this trait or this could be improved by intermating of superior genotypes of segregating population from recombination breeding. Prithviraj *et al.* (2017), Kant *et al.* (2021), Ahmed *et al.* (2022) also found similar results.

Moderate heritability coupled with moderate genetic advance as percent mean was exhibited by plant height (h^2_b = 58.20 %, GAM = 10.43%)

indicated that the traits were most likely to be controlled by additive gene action.

Low heritability coupled with low genetic advance as percent mean was exhibited by 1000-seed weight (h^2_b = 26.90%, GAM = 4.79 %) indicating this trait is governed by non-additive gene effects (dominance and/or epistasis) and highly influenced by environmental effects or a combination of these two variables. Hence direct selection for such trait would be ineffective. This was similar with the findings of Tripathy *et al.* (2016) and Patel *et al.* (2022).

By and large, it could be concluded that high to moderate estimates of GCV and PCV and high heritability with high genetic advance as per cent of mean were observed for seed yield per plant, number of capsules per plant and number of primary branches per plant indicating that the variation in the above characters is most likely due to additive gene effects and thus provides greater scope for improvement of these traits in sesame.

4.5 GENETIC DIVERGENCE

In any crop improvement programme, assessment of genetic diversity is must for identifying potential parents for hybridization. Diverse parents are expected to yield high frequency of heterotic hybrids in addition to generating a broad spectrum of variability in segregating generations. D^2 statistics is a useful multivariate analysis tool for measuring the genetic diversity in germplasm collection.

The data collected on 10 yield and yield contributing characters in 43 sesame genotypes were subjected to Mahalanobis D^2 statistics and the results are furnished here under.

4.5.1 Mahalanobis D^2 values

To estimate D^2 values, correlated means of 10 characters of 43 genotypes of sesame were transformed into standardized uncorrelated character using pivotal condensation method ($Y_1 – Y_2$). It measures degree of

diversification and determines the relative contribution of each component character to total diversification. The statistical differences (D^2) between pairs of genotypes were obtained as the sum of squares of the differences between the pairs of corresponding uncorrelated values of any two genotypes considered at a time. Thus, D^2 values were obtained for all possible 903 pairs [43 (43-1)/2] of genotypes.

4.5.2 Clusters Composition

The 43 sesame genotypes were grouped into seven distinct non over lapping clusters by using Tocher's method (Rao, 1952) and the distribution of genotypes into various clusters is presented in the Table 4.6 and illustrated in Fig 4.2. Cluster I was the largest comprising 25 genotypes followed by cluster II with 10 genotypes, cluster IV with 4 genotypes, and cluster III, cluster V, cluster VI, cluster VII were mono genotypic indicating divergence among the genotypes. Cluster I consisted maximum 25 genotypes indicating that the genotypes had narrow genetic divergence among them. The similarity in the base population, from which they had been evolved, might be the cause of genetic uniformity (Srikanth *et al.*, 2022). However, the unidirectional selection potential for one particular trait or a group of linked traits in several places may produce similar phenotypes which can be aggregated into one cluster irrespective of their geographic origin.

4.5.3 Intra and Inter-Cluster Average Distance

The intra and inter cluster distance (D^2) among seven clusters were presented in the Table 4.7 and Fig. 4.3. Based on the persual of results inter cluster distances were higher than intra cluster distance indicating the presence of wider genetic diversity between the clusters rather than with in the clusters. The intra cluster distances were ranged from 0.00 to 16.92. Among seven clusters, cluster II had the maximum intra cluster distance (16.92) indicating presence of wide genetic diversity among the genotypes present within this cluster, followed by cluster I (14.38) and cluster IV (13.54), indicating the presence of genetic diversity among the genotypes within these

Table 4.6 Distribution of 43 sesame genotypes into clusters based on Tocher's method

Cluster No.	No. of Genotypes	Genotypes
I	25	YLM-66, VRI-4, G.Til-10, YLM-146, Hima, RT-103, RT-46, RT-390, JTS-8, TKG-306, RT-127, RT-346, JCS-3603, TMV-7, YLM-17, JCS-2454, TKG-21, Swetha til, TKG-55, Gouri, RT-125, RT-351, G.Til-6, Rajeswari, TKG-308
II	10	G.Til-1, G.Til-2, E-8, G.Til-5, G.Til-3, TMV-4, VRI-2, Madhavi, Paiyur-1, Pratap
III	1	G.Til-4
IV	4	Chandana, CUMS-17, RT-54, Nirmala
V	1	YLM-11
VI	1	TKG-22
VII	1	JCS-1020

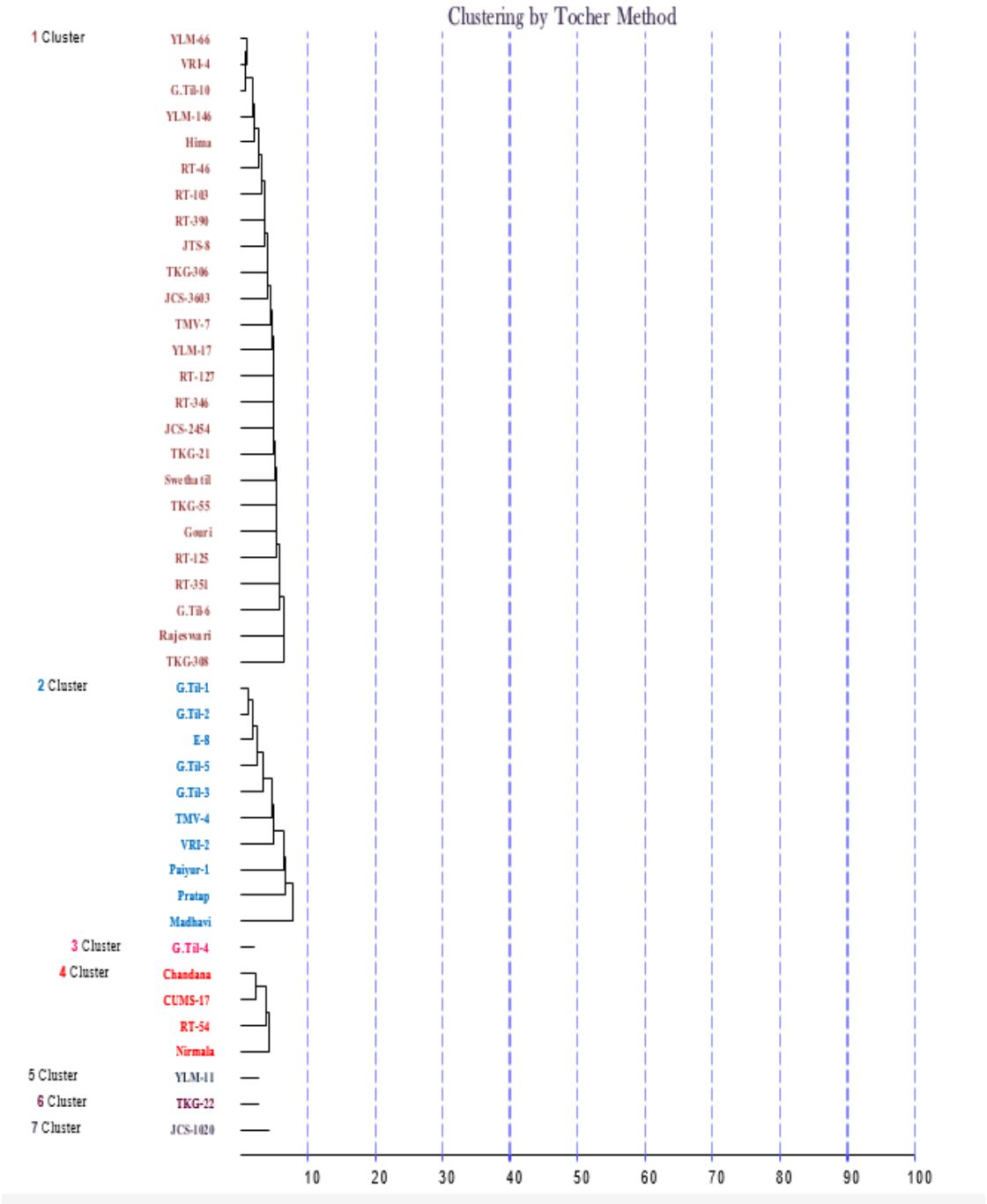


Fig.4.2. Distribution pattern of 43 sesame genotypes by Tocher's method

clusters, while intra cluster distance for cluster III, V, VI and VII was null as they are mono genotypic clusters. Genotypes grouped in the same cluster presumably differ little from one another as the aggregate of characters measured. Similar results were reported by Srikanth *et al.* 2022, Tesfaye *et al.* 2021, Uikey *et al.* 2022 and Yadav *et al.* 2022.

The maximum inter cluster distance was observed between cluster IV and VII (62.97) followed by cluster III and IV (54.08), cluster IV and VI (51.76), cluster V and VII (50.96) and cluster II and VII (50.88). Thus, selection of genotypes from these clusters which are quite divergent from each other can be used for hybridization programme which are expected to give high heterotic response resulting in better recombinants or desirable combinations for development of useful genetic stocks or varieties. Whereas the minimum inter cluster distance noted between cluster I and III (18.71) suggested that the genetic constitution of genotypes in cluster I was in close proximity with the genotype in the cluster III. Hence, selection of parents from these clusters is not effective due to narrow genetic base. Arpitha *et al.* (2019) and Ramprasad *et al.* (2019) reported similar results.

Clustering pattern revealed that genotypes from different regions fall into a one cluster which could be explained by the presence of unidirectional selection pressure for development of the genotypes which made them genetically similar as compared to their parents. The genotypes from the same origin may be present in same cluster or in different clusters (Tesfaye *et al.,* 2021). Looking at the pattern of distribution in different clusters, it appeared that geographical distance between the genotypes had no relation with the genetic divergence as the genotypes from same source had fallen into different clusters as well as the same cluster contained varieties from different sources. Therefore, it can be concluded that the selection of parents for hybridization should not be based on geographical diversity, but it should have a base of both geographical origin as well as genetic divergence.

Table 4.7 Average Inter (above diagonal) and Intra (diagonal) cluster distances (D^2 values) for seven clusters of 43 sesame genotypes

Cluster	I	II	III	IV	V	VI	VII
I	14.38 (3.79)	29.69 (5.45)	18.71 (4.33)	33.08 (5.75)	19.78 (4.45)	25.04 (5.00)	29.46 (5.43)
II		16.92 (4.11)	25.16 (5.02)	46.63 (6.83)	50.76 (7.12)	30.78 (5.55)	50.88 (7.13)
III			0.00 (0.00)	54.08 (7.35)	28.14 (5.30)	26.9 (5.19)	22.49 (4.74)
IV				13.54 (3.68)	46.96 (6.85)	51.76 (7.19)	62.97 (7.94)
V					0.00 (0.00)	37.72 (6.14)	50.96 (7.14)
VI						0.00 (0.00)	37.89 (6.16)
VII							0.00 (0.00)

Note: Figures in the parenthesis indicate D values

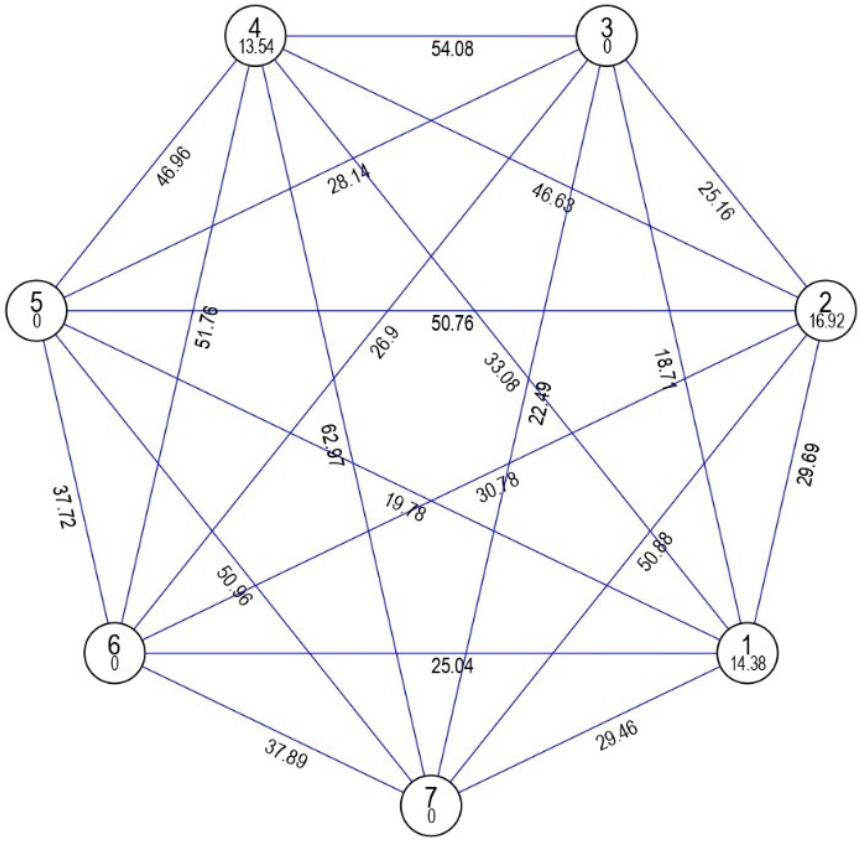


Fig. 4.3. Intra (D) and Inter (D^2) cluster distances representation by Tocher's diagram

4.5.4 Cluster Means for Various Characters

Cluster mean values were estimated for 10 quantitative traits are presented in Table 4.8. Cluster mean for days to 50 % flowering ranged from 32.33 days in cluster VI to 43.00 days in cluster V with a general mean of 38.48 days. Early genotypes were found in cluster VI and late genotypes were in cluster V.

Days to maturity ranged from 79.00 days (cluster V) to 88.83 days (cluster II) with a general mean of 83.78 days. Early genotype was found in cluster V and late genotypes were found in cluster II.

The trait plant height was maximum in cluster III (86.67 cm) while minimum in cluster I (99.60 cm). The cluster I, cluster VII, cluster V and cluster IV exceeded the general mean of plant height (94.92 cm).

Number of primary branches per plant was lowest in cluster VII (1.33), while it was highest in cluster V (5.33). Highest cluster mean than general mean (3.76) was recorded in cluster V, cluster IV, cluster VI, cluster 1 and II.

Number of capsules per plant ranged from 62.40 in cluster III to 80.80 in cluster V with general mean of 71.90. Highest cluster mean than general mean was recorded by cluster V, cluster IV, cluster II and cluster 1.

Capsule length was minimum in cluster III (2.71 cm) and maximum in cluster VI (3.21 cm). Cluster VI, cluster I, cluster VII and cluster II surpassed the general mean (2.96 cm).

Cluster mean for number of seeds per capsule ranged from 65.67 in cluster III and cluster V to 90.60 in cluster VI with general mean of 72.57. Highest cluster mean than general mean was recorded in cluster VI and cluster IV.

Cluster mean for 1000-seed weight was lowest in cluster V (2.90 g) and highest in cluster III (3.53g) with general mean of 3.13 g. Clusters III and VII recorded higher values for 1000-seed weight than general mean.

Oil content differed from 35.71 % in cluster IV to 47.88% in cluster III. Cluster. III, cluster VII, cluster VI, cluster V and II exceeded the general mean (44.96 cm) for oil content.

Seed yield per plant ranges between 9.70 g in cluster V to 15.31 g in cluster II with general mean of 11.88 g. Clusters II, cluster VI and cluster I recorded higher values for grain yield per plant than general mean (11.88g).

From the foregoing results, it was observed that cluster mean for different characters showed considerable differences between the clusters for all the characters (Table 4.8). The lower mean values are desirable for days to 50% flowering and days to maturity trait. Cluster V and Cluster VII exhibited lower mean values for days to 50 % flowering and days to maturity respectively. Hence, genotypes selected from this could be used as parents in hybridization programme for developing short duration varieties. Cluster V recorded lowest mean values for days to 50 % flowering and highest mean values for number of primary branches per plant, number of capsules per plant; cluster III for 1000-seed weight and oil content; Cluster VI for capsule length and number of seeds per capsule; Cluster II for days to maturity and seed yield per plant; Cluster I for plant height. Hence selection of these genotypes from these clusters would be more rewarding for development of high yielding sesame varieties along with high oil content and early duration.

Table 4.8 Cluster means for yield and yield attributing traits among 43 sesame genotypes

Cluster	Days to 50 % flowering	Days to maturity	Plant height (cm)	Number of primary branches per plant	Number of capsules per plant	Capsule length (cm)	Number of seeds per capsule	1000-seed weight (g)	Oil content (%)	Seed yield per plant (g)
I	39.03	83.05	99.60	4.10	72.30	3.03	69.21	3.11	44.72	13.15
II	39.83	88.83	94.77	3.85	78.60	2.97	72.33	3.12	45.95	15.31
III	40.33	84.33	86.67	3.13	62.40	2.71	65.67	3.53	47.88	10.53
IV	41.17	83.25	98.13	4.43	80.67	2.88	73.63	2.93	35.71	14.17
V	43.00	79.00	98.73	5.33	80.80	2.93	68.27	2.90	46.61	9.70
VI	32.33	85.00	87.50	4.13	60.07	3.21	90.60	2.93	46.73	9.83
VII	33.67	83.00	99.07	1.33	68.47	2.98	68.27	3.37	47.10	10.47
Mean	**38.48**	**83.78**	**94.92**	**3.76**	**71.90**	**2.96**	**72.57**	**3.13**	**44.96**	**11.88**

4.5.5 Relative Contribution of Each Character Towards Diversity

The selection and choice of parents mainly depend upon contribution of character towards divergence. The number of times that each of the 10 characters appeared in first rank and its respective per cent contribution towards diversity is presented in Table 4.9.

Table 4.9 Percent contribution of various characters towards genetic divergence in 43 sesame genotypes

S.No.	Character	No. of times ranked 1st	Contribution (%)
1	Days to 50% flowering	65	7.20%
2	Days to maturity	322	35.66%
3	Plant height (cm)	39	4.32%
4	Number of primary branches per plant	106	11.74%
5	Number of capsules per plant	58	6.42%
6	Capsule length (cm)	56	6.20%
7	Number of seeds per capsule	40	4.43%
8	1000 seed weight (g)	2	0.22%
9	Oil content (%)	184	20.38%
10	Seed yield per plant (g)	31	3.43%

Among all the characters studied, days to maturity (35.66 %) contributed maximum towards genetic diversity ranking 322 times first followed by oil content (20.38 %) ranking 184 times first, number of primary branches per plant (11.74 %) ranking 106 times, days to 50 % flowering (7.20 %) ranking 65 times and number of capsules per plant (6.42 %) ranking 58 times. The characters *viz.,* capsule length (6.20 %), number of seeds per capsule (4.43 %), plant height (4.32 %), seed yield per plant (3.43 %) contributed less towards genetic diversity, while 1000-seed weight had negligible contribution towards the genetic diversity. Days to maturity followed by oil content, number of primary branches per plant and days to 50 % flowering contributed relatively maximum towards the total divergence.

Hence, emphasis should be given in hybridization programme to generate large variability thus it provides immense scope for the improvement of yield.

The results were in consonance with findings of Rajitha *et al.* (2018), Sirisha *et al.* (2020), Srikanth *et al.* (2022) for days to maturity; Rajitha *et al.* (2018), Jamir *et al.* (2020) for oil content; Suganthi *et al.* (2017) for number of primary branches per plant; Begum *et al.* (2017), Gogoi *et al.* (2018) for number of capsules per plant; Tanwar and Bisen (2018) for capsule length; Gogoi *et al.* (2018) for plant height and Sasipriya *et al.* (2023) for 1000-seed weight.

It is assumed that maximum amount of heterosis will be manifested in cross combinations involving the parents from the most divergent clusters and complementarity for traits of interest will lead to accumulation of genes in a single variety. Hence, the genotypes, Paiyur-1 from cluster II, followed by JCS-1020 from cluster VII, Nirmala from cluster IV, G.Til-4 from cluster III and Hima from cluster I could be selected as a parents in future hybridization programme as they expressed high *per se* performance for more number of traits, maximum inter cluster distance and complementarity of traits of interest.

4.6 ASSESSMENT OF MOLECULAR DIVERSITY

The knowledge of genetic variation present in the base population of sesame will provide critical information for better management strategy for crop improvement. Diversity analysis based only on morphological characters are prone to environmental bias due to environmental influences and complex genetic structure of different morphological traits (Banerjee and Kole 2009). So combination of both morphological and molecular study would be more preferable. The genetic diversity in sesame has been thus far detected by universal markers such as random amplified polymorphic DNA (RAPD) (Arriel *et al.*, 2007), amplified fragment length polymorphisms (AFLPs) (Lauretin *et al.*, 2007), inter simple sequence repeats (ISSRs) (Parsaeian *et al.*, 2011), genome sequence-simple sequence repeats (gSSRs) (Dossa *et al.*,

2016), chloroplast SSR (cpSSRs) (Sehr *et al.*, 2016) and expressed sequence tag-SSRs (EST-SSRs) (Badri *et al.*, 2014). SSRs are considered an ideal marker choice for assessing the genetic diversity and germplasm characterization considering their high-fidelity, co-dominant nature, chromosome specificity, high polymorphism and reproducibility (Bhattacharjee *et al.*, 2018).

4.6.1 Number and Frequency of alleles

Thirty four sesame specific SSR markers (Simple Sequence Repeats) developed by hybridization method were used to characterize and assess genetic diversity among 43 genotypes of sesame. For each SSR locus, sizes of the alleles were estimated for all the 43 genotypes and scored in the form of a binary matrix where '1' represented the presence of a band and '0' denoted its absence. Pair-wise genetic similarity (GS) was calculated among 43 genotypes using Jaccard's dissimilarity coefficient. The PIC and the number of alleles of the polymorphic markers are mentioned in Table 4.10.

Among 34 markers tested, only nine showed polymorphism (SEM 12-65, Si-14 and SEM 12-14, Si-15, Si-3, Sa-08, SSR 212, SSR 217, SSR 34) and remaining 25 are found to be monomorphic. Yepuri *et al.* (2013) and Kumari (2017) also reported similar results of very less number of polymorphic SSR markers in sesame. The polymorphic percentage is 26 % which is likely similar to the 29.4% reported by Ramprasad *et al.* (2017). In contrary Wu *et al.* (2014), Dossa *et al.* (2016) and Sasipriya *et al.* (2020) reported 50-60% polymorphism.

The primer SEM 12-65 have scored 4 alleles compared to other primers which scored 3 alleles (Si-14 and SEM 12-14) and 2 alleles (Si-15, Si-3, Sa-08, SSR212, SSR217, SSR34). In contrary, Kumar and sharma (2011) reported 4 alleles for Sa-08 primer. The average number of alleles obtained is 2.4 which is very similar to 2.8 obtained Ramprasad *et al.* (2017), lower than 3.11 reported by Badri *et al.* (2014), lower than the 3.37 reported by Pandey *et al.* (2015).

Table 4.10 Details of the number of alleles, major allele frequency, allele size and Polymorphism Information Content (PIC) of SSR markers

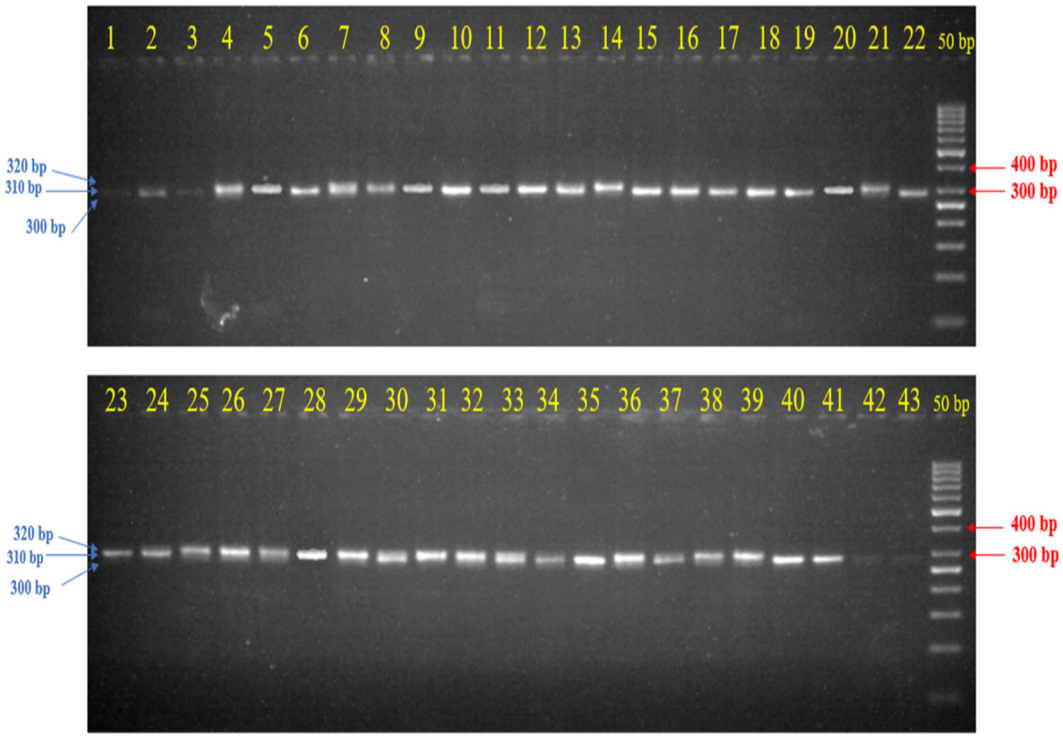
S. No.	Primer	No. of alleles	Major allele frequency	Allele size (bp)	PIC
1	Si-15	2	0.88	170 and 180	0.205
2	Si-14	3	0.79	130, 135 and 140	0.339
3	Si-3	2	0.79	210 and 220	0.330
4	Sa-08	2	0.95	135 and 150	0.088
5	SEM 12-14	3	0.83	300, 310 and 320	0.285
6	SSR 212	2	0.97	320 and 330	0.045
7	SSR 217	2	0.53	240 and 260	0.497
8	SEM12-65	4	0.60	180,200,220 and 230	0.549
9	SSR 34	2	0.90	220 and 230	0.168
	Average	**2.4**			**0.279**

4.6.2 Polymorphism information content of SSR markers

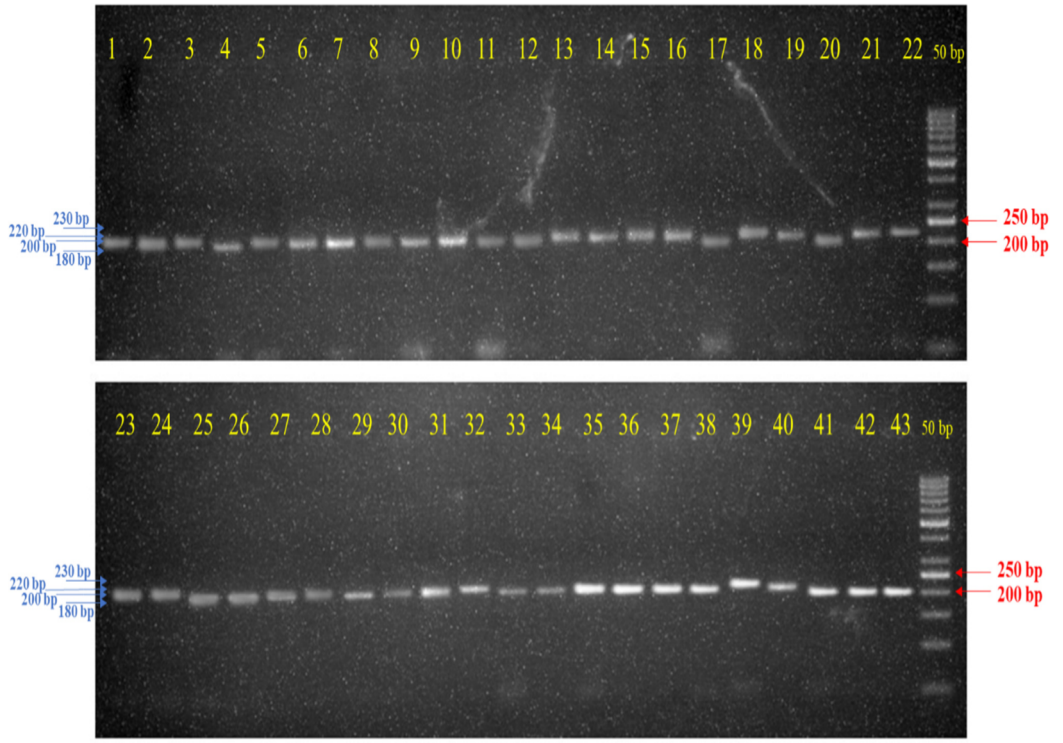
Polymorphism information content (PIC) provides an estimate of the discriminatory power of the marker considering both the number and relative frequency of the alleles and the values ranged from 0 (monomorphic) to 1 (Highly polymorphic). In this study, PIC values in polymorphic markers ranged from 0.045 (SSR 212) to 0.549 (SEM 12-65) with an average of 0.279. PIC values in the present study were lower than the previous reports of Evangelia *et al.* (2021) who reported PIC value of 0.82. This might be due to limited number of genotypes screened and varietal difference.

Based on the level of polymorphism detected by individual marker, four most informative SSR markers namely SEM 12-65 (0.549), SSR 217 (0.497), Si-14 (0.339) and Si-3 (0.330) were identified which displayed very high PIC values. This set of markers had the enormous potential to identify most of the sesame genotypes under study. In contrast, PIC of SSR 212 was very low indicating a less discriminatory power of this marker in distinguishing the genotypes.

SEM-12-14



SEM-12-65



(1-pratap, 2-TKG21, 3-RT54, 4-RT46, 5-JCS2454, 6-CUMS17, 7-JTS8, 8-RT-351, 9-Chandana, 10-VRI2, 11-RT125, 12-RT-346, 13-RT-390, 14-G.Til2, 15-Hima, 16-RT127, 17-Gouri, 18-YLM11, 19-YLM1, 20-G.Til1, 21-G.Til3, 22-YLM66, 23-YLM146, 24-Madhavi, 25-G.Til4, 26-G.Til5, 27-G.Til6, 28-G.Til10, 29-RT103, 30- Nirmala, 31-Swetha til, 32-Rajeswari, 33-TKG22, 34-TKG55, 35-TKG306, 36-TKG308, 37-E8, 38-JCS1020, 39-JCS3603, 40-TMV4, 41-TMV7, 42-VRI4, 43-Paiyur1)

Plate 14: Amplification profile of SEM 12-14 and SEM 12-65 SSR Markers in 43 sesame genotypes

4.6.3 Construction of DNA barcode

The polymorphic SSR markers were used to develop varietal-specific DNA fingerprints for all the 43 sesame varieties. The alleles of the polymorphic markers were assigned with codes *i.e.,* A, B, C and D based on their allele sizes in ascending order. The pattern of allele codes of these polymorphic markers were used to depict the DNA fingerprints. The differences in the pattern with respect to even a single allele code could distinguish one variety from the other. Construction of barcodes suggested by Gao *et al.* (2009) was applied in sesame by Bhattacharjee *et al.* (2018). Employing these codes of the polymorphic markers, 17 of 43 sesame varieties could be unambiguously distinguished and other 26 varieties could not be distinguished as they were possessing similar allele codes (DNA fingerprints) possibly due to a limited number of polymorphic markers.

Out of 17 distinguished genotypes, six genotypes G.Til-1, G.Til-3, JCS 2454, RT-54, TKG-306 and YLM-66 were highly distinguished at 6 allele level. Whereas seven genotypes TMV-7, Chandana, GTil-2, RT-46, RT-390, TKG-55 and TMV-4 were differentiated at 5 allele level and the remaining four genotypes were discriminated at one allelic level. The marker SEM 12-65 produced unique allele which alone differentiated JCS-3603 and YLM-11. Similarly, Si-14 differentiated RT-54 at unique allele level. The representation of varietal-specific fingerprints is given in the Fig. 4.4.

Similar allelic code were exhibited by 26 genotypes which was represented in Fig. 4.5. The varieties *viz*., RT-351, VRI-2, RT-125, RT-346, Gouri, Rajeswari produced similar allele code; YLM-146, Madhavi, TKG-22, VRI-4, Paiyur-1 showed similar allele code; RT-103, Nirmala, Swetha til showed similar allele code; Pratap, TKG-21, CUMS-17 showed similar allele code; JTS-8, G.Til-6, G.Til-10 showed similar allele code; Hima, RT-127 produced similar allele code; G.Til-4, G.Til-5 showed similar allele code and TKG-308, E-8 showed similar allele code.

S.No	Genotypes	Si-15	Si-14	Si-3	Sa-8	SEM12-14	SSR 212	SSR 217	SEM12-65	SSR 34	Allele codes
1	Chandana	B	B	B	A	B	A	B	B	A	BBBABABBA
2	G.Til-1	A	A	B	B	B	A	B	B	A	AABBBABBA
3	G.Til-2	B	B	B	B	C	A	B	C	B	BBBBCABCB
4	G.Til-3	A	A	B	B	B	A	A	C	A	AABBBAACA
5	JCS-1020	B	B	B	B	B	B	A	C	A	BBBBBBACA
6	JCS-2454	A	B	A	B	B	A	B	B	A	ABABBABBA
7	JCS-3603	B	B	B	B	B	A	A	D	A	BBBBBAADA
8	RT-390	A	B	B	B	B	A	B	C	B	ABBBBABCB
9	RT-46	B	B	A	B	B	A	B	A	A	BBABBABAA
10	RT-54	B	C	A	B	B	A	B	B	A	BCABBABBA
11	TKG-306	A	B	B	B	A	A	A	C	A	ABBBAAACA
12	TKG-55	B	B	B	A	B	A	A	B	A	BBBABAABA
13	TMV-4	B	B	B	B	A	A	A	C	A	BBBBAAACA
14	TMV-7	B	B	B	B	A	A	A	B	A	BBBBAAABA
15	YLM-11	B	B	B	B	B	A	B	D	A	BBBBBABDA
16	YLM-17	B	B	B	B	B	A	B	C	A	BBBBBABCA
17	YLM-66	B	A	B	B	A	A	A	C	A	BABBAAACA

The letters A, B, C and D are the alleles of specific marker recorded by the genotype

Allele code key

Allele code	Si-15	Si-14	Si-3	Sa-8	SEM12-14	SSR 212	SSR 217	SEM12-65	SSR 34
A	170	130	210	135	300	320	240	180	220
B	180	135	220	150	310	330	260	200	230
C		140			320			220	
D								230	

Fig. 4.4. DNA fingerprints for 17 genotypes of sesame having different allelic code

S.No	Genotypes	Si-15	Si-14	Si-3	Sa-8	SEM12-14	SSR 212	SSR 217	SEM12-65	SSR 34	Allele codes
1	Pratap	B	B	A	B	B	A	B	B	A	BBABBABBA
2	TKG-21	B	B	A	B	B	A	B	B	A	BBABBABBA
3	CUMS-17	B	B	A	B	B	A	B	B	A	BBABBABBA
4	JTS-8	B	B	A	B	B	A	A	B	A	BBABBAABA
5	G.Til-6	B	B	A	B	B	A	A	B	A	BBABBAABA
6	G.Til-10	B	B	A	B	B	A	A	B	A	BBABBAABA
7	RT-351	B	B	B	B	B	A	B	B	A	BBBBBABBA
8	VRI-2	B	B	B	B	B	A	B	B	A	BBBBBABBA
9	RT-125	B	B	B	B	B	A	B	B	A	BBBBBABBA
10	RT-346	B	B	B	B	B	A	B	B	A	BBBBBABBA
11	Gouri	B	B	B	B	B	A	B	B	A	BBBBBABBA
12	Rajeswari	B	B	B	B	B	A	B	B	A	BBBBBABBA
13	Hima	B	B	B	B	B	A	B	C	B	BBBBBABCB
14	RT-127	B	B	B	B	B	A	B	C	B	BBBBBABCB
15	YLM-146	B	B	B	B	B	A	A	B	A	BBBBBAABA
16	Madhavi	B	B	B	B	B	A	A	B	A	BBBBBAABA
17	TKG-22	B	B	B	B	B	A	A	B	A	BBBBBAABA
18	VRI-4	B	B	B	B	B	A	A	B	A	BBBBBAABA
19	Paiyur-1	B	B	B	B	B	A	A	B	A	BBBBBAABA
20	RT-103	B	A	B	B	B	A	B	B	A	BABBBABBA
21	Nirmala	B	A	B	B	B	A	B	B	A	BABBBABBA
22	Swetha Til	B	A	B	B	B	A	B	B	A	BABBBABBA
23	TKG-308	B	B	B	B	B	A	A	C	A	BBBBBAACA
24	E-8	B	B	B	B	B	A	A	C	A	BBBBBAACA
25	G.Til-4	B	A	B	B	C	A	A	A	A	BABBCAAAA
26	G.Til-5	B	A	B	B	C	A	A	A	A	BABBCAAAA

The letters A, B ,C and D are the alleles of specific marker recorded by the genotype. The varieties having similar allele code were represented in the same colour

Allele code key

Allele code	Si-15	Si-14	Si-3	Sa-8	SEM12-14	SSR 212	SSR 217	SEM12-65	SSR 34
A	170	130	210	135	300	320	240	180	220
B	180	135	220	150	310	330	260	200	230
C		140			320			220	
D								230	

Fig. 4.5. DNA fingerprints for 26 genotypes of sesame having similar allelic code

4.6.4 Development of varietal-specific fingerprints using both DUS descriptors and molecular markers

Varietal specific DNA fingerprints revealed that, among the 34 SSR primers used, nine primers were polymorphic and the remaining 25 were monomorphic. These nine primers were found to be useful in fingerprinting of 17 varieties whereas in combination of nine SSR markers and 12 DUS descriptors (petal colour, petal hairiness, branching pattern, stem hairiness, leaf lobes, leaf size, leaf serration of margins, capsule hairiness, capsule shape, capsule number per leaf axil, capsule arrangement and seed colour) all the genotypes could be discriminated. Hence, it could be recommended that in spite of using either morphological or molecular markers alone for characterization and identification of sesame genotypes, it is better to use both phenotypic and molecular descriptors for identification of varieties.

4.6.5 Genetic diversity analysis

The genetic relationship among the 43 sesame genotypes is presented in the form of a dendrogram constructed by UPGMA cluster analysis (Unweighted Pair Group Method with Arithematic mean) using Jaccard's coefficient. The cluster analysis of the nine SSR markers was represented in the Table 4.11. The dendrogram grouped the 43 genotypes into three clusters and represented in the Fig. 4.6.

The Cluster-I had 22 genotypes and subdivided into IA and IB. IA with 16 genotypes and IB with 6 genotypes. The genotypes YLM-11 and YLM-17 were grouped into cluster-I. Surprisingly, these genotypes were the derivatives of the cross (Vinayak × Kanak). In the similar way TKG-306, YLM-66 and JCS-1020 were grouped in cluster-I along with their parents TKG-22, YLM-17 and E-8, respectively indicating the genetic similarity among the genotypes. Yepuri *et al.* (2013) reported most of the cultivated varieties like Swetha til, Rajeswari, RT-54 fell into one cluster.

Table 4.11 Grouping of 43 sesame genotypes into three clusters based on Jaccard's dissimilarity coefficient using DARwin software

Cluster No.	Sub clusters	No. of genotypes	Name of genotypes
Cluster I	IA	16	TMV-4, TKG-306, YLM-66, G.Til-3, G.Til-5, G.Til-4, E-8, TKG-308, JCS-1020, RT-127, Hima, G.Til-2, RT-390, YLM-17, YLM-11, JCS-3603
	IB	6	Paiyur-1, Madhavi, TKG-22, VRI-4, YLM-146, TMV-7
Cluster II	IIA	10	RT-125, VRI-2, Gouri, RT-346, RT-351, Rajeswari, Nirmala, RT-103, Swetha til, G.Til-1
	IIB	9	TKG-21, Pratap, CUMS-17, JCS-2454, RT-54, RT-46, G.Til-6, JTS-8, G.Til-10
Cluster III	-	2	TKG-55, Chandana

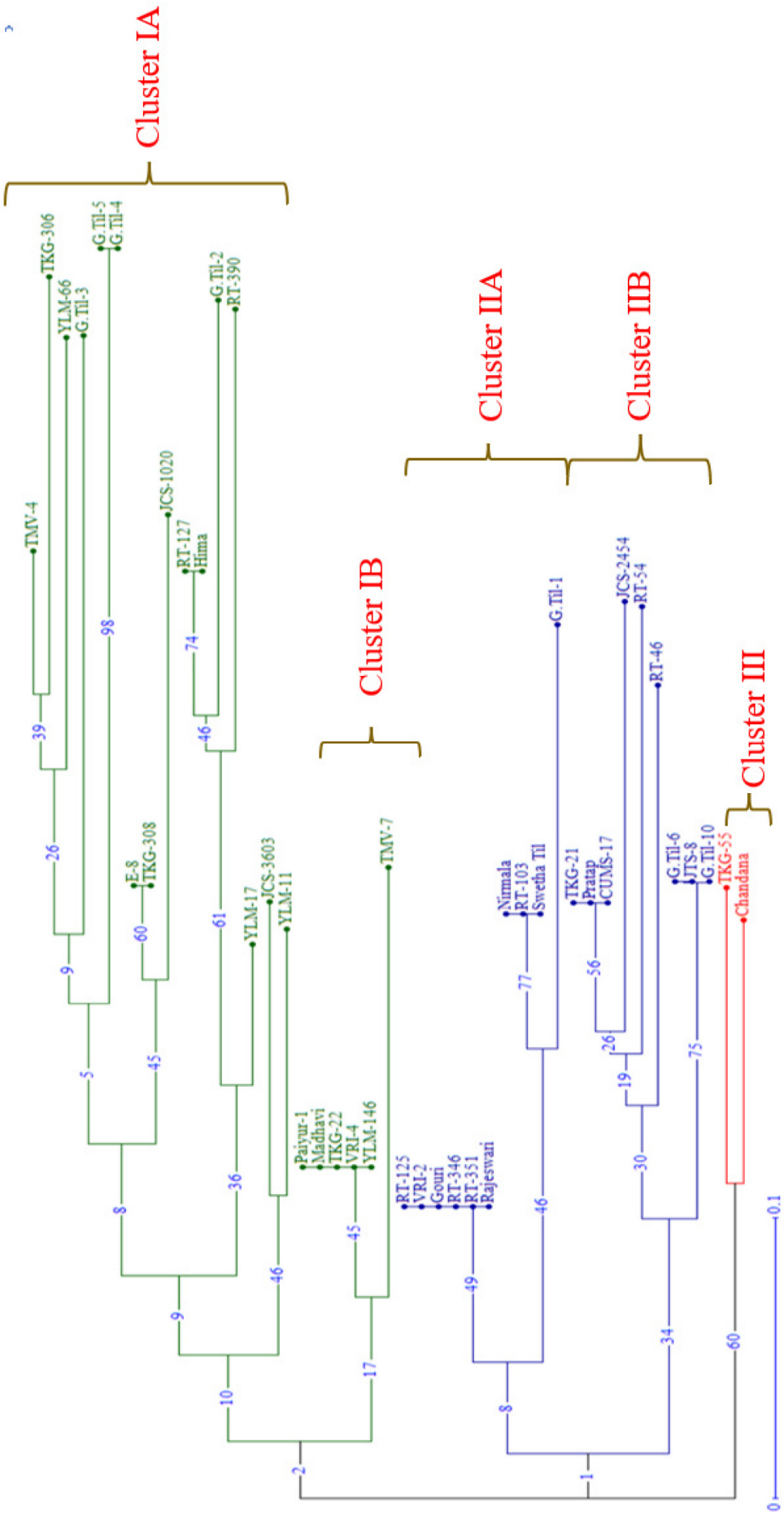


Fig. 4.6. UPGMA cluster analysis for 43 sesame genotypes

Cluster-II had 19 genotypes and was sub divided into IIA and IIB. Cluster-IIA consisting of 10 genotypes and IIB with 9 genotypes. Two genotypes *i.e.,* TKG-21 and RT-46 along with their common parent Punjab.Til-1were grouped together in cluster-II. Further, cluster-III had only two genotypes *i.e.,* TKG-55 and Chandana.

The lack of association between geographical origin and genetic diversity was observed among the varieties. This was attributed to free exchange of genotypes from one region to another and also may be due to the character constellation that might have been practiced in several regions resulting in segregation of genotypes irrespective of their geographic region. Similar results were reported earlier by Ramprasad *et al.* (2017).

4.6.6 Development of QR Code System

QR codes scan can be used as labels for tagging the breeding material both in field and laboratory. Any user can access the information on QR code by scanning the QR codes using the hand-held data capture devices such as barcode or QR code scanner or by using smartphones, tablet or laptop with a camera, using freely available software.

In the present investigation, descriptor notes of the 12 essential DUS traits in combination with allele codes of the SSR markers were converted to Quick Response (QR) codes using online tool (available at qrcode-generator.com org). The QR (Quick Response) codes generated for all the 43 genotypes using combination of DUS traits and allele codes are represented in the Fig. 4.7.

Pratap

TKG-21

RT-54

RT-46

JCS-2454

CUMS-17

JTS-8

RT-351

Chandana

VRI-2

RT-125

RT-346

RT-390

G.Til-2

Hima

RT-127

Gouri

YLM-11

YLM-17

G.Til-1

G.Til-3

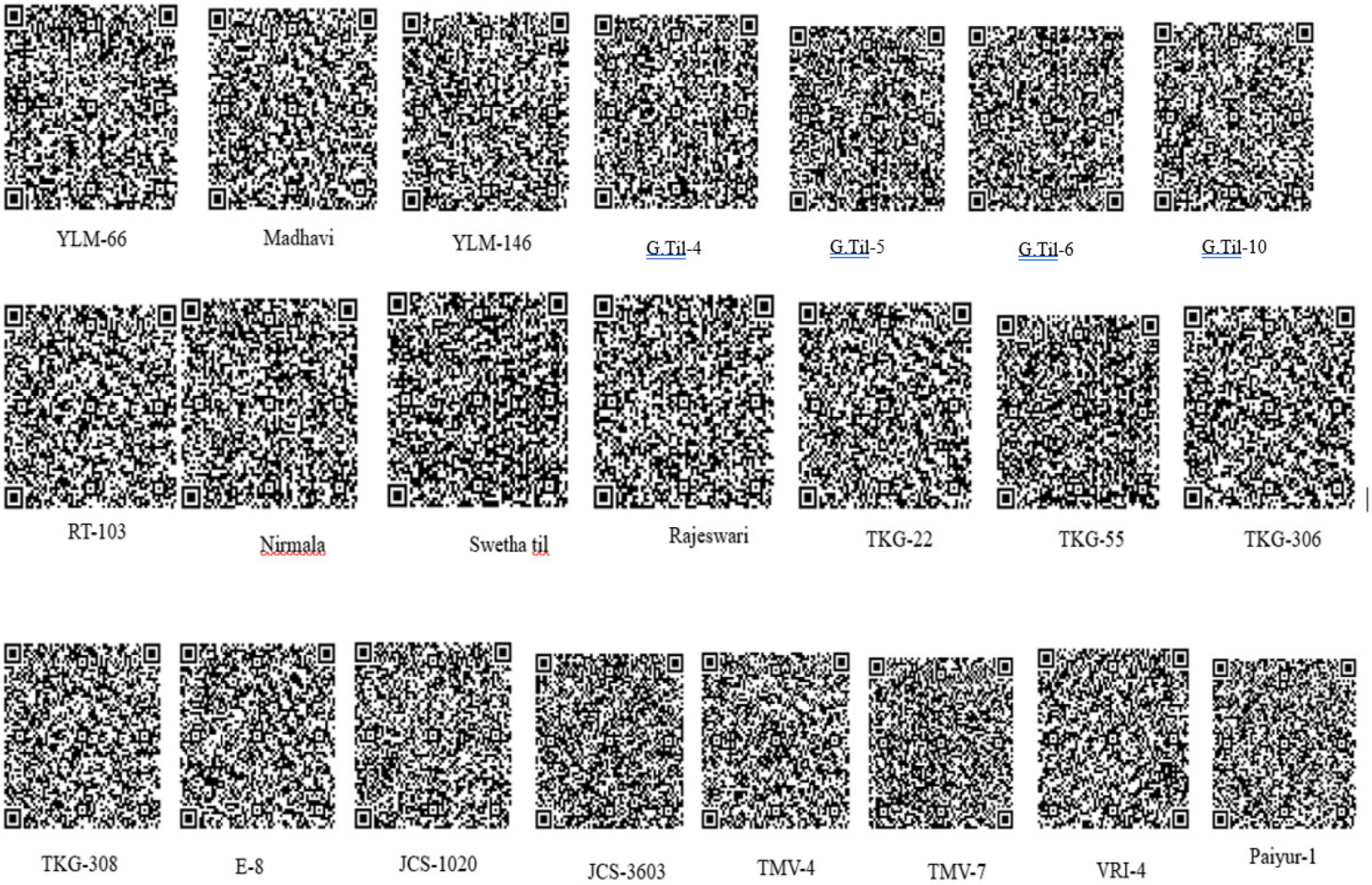


Fig. 4.7. QR codes generated for all the 43 sesame genotypes using combined DUS and allele codes

Chapter - V

Summary & Conclusions

Chapter-V

SUMMARY AND CONCLUSIONS

The present work titled “DUS characterization and molecular diversity analysis in sesame (*Sesamum indicum* L.)” was conducted at Regional Agricultural Research Station, Tirupati during *Rabi*, 2022-23 in a Randomized Block Design (RBD) with three replications. In this study, 43 genotypes of sesame were comprehensively characterized for 20 DUS traits and genetic parameters (variability, heritability and genetic advance), genetic divergence for 10 quantitative traits *viz*., days to 50 % flowering, days to maturity, plant height, number of primary branches per plant, number of capsules per plant, capsule length, number of seeds per capsule, 1000-seed weight, seed yield per plant and oil content were estimated and varietal specific DNA fingerprints were developed using both DUS descriptors and DNA markers.

DUS characterization of 43 genotypes of sesame for 20 DUS traits revealed the existence of abundant diversity for the characters studied and the predominance of medium duration flowering; light purple petal colour; sparse petal hairiness; medium plant height; profuse plant branching; top branching pattern; sparse stem hairiness; deeply lobed leaves; medium size of leaf; strong serration of leaf; sparse capsule hairiness; four locule number per capsule; broad oblong capsule shape; one capsule number per leaf axil; alternate capsule arrangement; long capsule length; medium days to maturity; white seed colour; medium 1000-seed weight and medium oil content were noted. The variation observed among the cultivars could be better utilized in the selection of genotypes based on trait specific requirement in future breeding programmes.

The analysis of variance (ANOVA) revealed that mean sum of squares were highly significant for all the characters *viz*., days to 50% flowering, days to maturity, plant height (cm), number of primary branches per plant, number of capsules per plant, capsule length (cm), number of seeds per capsule, 1000-

seed weight (g), seed yield per plant (g), oil content (%) revealing the presence of substantial magnitude of genetic variability among the genotypes.

Based on the estimates of *per se* performance, several genotypes exhibited high mean performance for more than one character. The genotype Hima exhibited superiority for days to maturity, plant height, number of primary branches per plant, number of capsules per plant and seed yield. Similarly, Paiyur-1 displayed higher performance for number of primary branches per plant, number of capsules per plant, number of seeds per capsule and seed yield per plant. Further, the genotype JCS-1020 displayed high mean values for days to 50 % flowering followed by 1000-seed weight and oil content. Hence, these genotypes (Hima, Paiyur-1 and JCS 1020) could be utilized as a potential donors for improvement of seed yield along with high oil content.

A perusal of results on variability revealed that high to moderate estimates of GCV and PCV, high heritability and high genetic advance as percent mean was recorded for seed yield per plant, number of primary branches per plant and number of capsules per plant which indicated that the variation in the above characters was most likely due to additive gene effects. Thus, direct selection for these characters may be effective in selection of elite genotypes for hybridization in sesame. High heritability coupled with moderate genetic advance as percent mean were exhibited by oil content, days to 50% flowering, capsule length, number of seeds per capsule and plant height representing that these traits were most likely to be controlled by additive gene action. Hence, direct and simple selection for these could be exercised due to fixable additive gene effects.

The results of divergence studies through D^2 statistics noted that, the experimental material was assigned into 7 clusters, revealing the existence of substantial diversity among the genotypes for the traits studied. Cluster V recorded highest mean values for days to 50 % flowering, number of primary branches per plant and number of capsules per plant. Similarly, highest mean

values for, 1000-seed weight and oil content were recorded in cluster III whereas cluster VI recorded the highest mean values for capsule length and number of seeds per capsule.

Maximum inter cluster distance was observed between cluster IV and VII followed by cluster III and IV, cluster IV and VI, cluster V and VII and cluster II and VII indicating high genetic diversity between the clusters. Hence, selection of genotypes from these clusters in crossing programs would provide high heterotic and transgressive segregants in desirable combinations for development of high yielding sesame varieties. It was also revealed that among all the characters studied, days to maturity contributed maximum towards genetic diversity followed by oil content, number of primary branches per plant, days to 50 % flowering and number of capsules per plant. The genotypes Paiyur-1 from cluster II, JCS-1020 from cluster VII, Nirmala from cluster IV, G.Til-4 from cluster III and Hima from cluster I could be selected as a parents in future breeding programme as they expressed high *per se* performance for more number of traits, maximum inter cluster distance and complementarity for more number of traits of interest.

Molecular profiling of 43 sesame genotypes revealed that out of 34 SSR markers used, 9 were polymorphic and generated alleles ranging from 2 to 4 with an average of 2.4 per locus. Polymorphism information content (PIC) values varied from 0.045 (SSR 212) to 0.549 (SEM 12-65) with an average of 0.279. The polymorphic percentage is 26%. Through DNA fingerprinting with 9 polymorphic SSR markers, 17 genotypes were distinguished whereas, by using both 12 DUS descriptors and alleles codes of nine polymorphic markers, all the 43 sesame varieties could be differentiated. The QR codes were generated for all the 43 genotypes using combination of DUS traits and allele codes. Therefore, both morphological and molecular diversity are important for the identification of varieties.

FUTURE LINE OF WORK:

- Based on *per se* performance, morphological diversity using D^2 statistic and UPGMA cluster analysis, the crosses Paiyur-1 × Pratap, JCS-1020 × Nirmala, G.Til-4 × Pratap and Hima × Nirmala could be suggested for future breeding programme for development of high yielding sesame varieties.

Literature Cited

LITERATURE CITED

Abate, M and Mekbib, F. 2015. Study on genetic divergence in low-altitude sesame (*Sesamum indicum* L.) germplasm of Ethiopia based on agro-morphological traits. *Advanced Studies in Agricultural, Biological and Environmental Sciences*. 2: 78-90.

Abhijatha, A., Madhukar, K and Arya, K. 2017. Evaluation of sesame (*Sesamum indicum* L.) genotypes to the shaded uplands of southern region. *International Journal of Current Microbiology and Applied Science.* 6(7): 332-339.

Ahmed, A.S.N., Das, S., Saharia, D.D., Sarma, M.K., Nath, M.P and Talukdar, J.C. 2022. Assessment of Genetic variability and Character Association for Morpho-physiological Attributes and Seed Yield in Sesame. In *Biological Forum-An International Journal*. 14: 425-430.

Amit, S., Ram, A., Nisha, K., Sangwan, O and Sheoran, R.K. 2015. Principal component and hierarchical cluster analyses for classification and categorization of sesame germplasm. *Research On Crops.* 16(2): 345-350.

Anandhi, K. 2015. Protection of plant varieties and farmer's rights (PPVFR) act: A review. *Current Advances in Agricultural Sciences (An International Journal)*. 7(2):101-105.

Arpitha, G.D., Manonmani, S., Viswanathan, P.L and Raveendran, M. 2019. Genetic diversity study in germplasm lines of sesamum (*Sesamum indicum* L.). *Electronic Journal of Plant Breeding*. 10(2): 772-777.

Arriel, N.H.C. Di Mauro, A.O. Arriel, E.F. S.H. Costa, M.M. Bárbaro, I.M. Muniz, F.R.S. Genetic divergence in sesame based on morphological and agronomic traits. *Crop. Breeding and Applied Biotechnology*. 2007. 7: 253–261.

Azeez, M.A., Olowookere, M.B., Animasaun, D.A and Bello, B.O. 2016. Utility of some floral characters in the assessment of genetic diversity in sesame (*Sesamum indicum* L.). *Acta agriculturae Slovenica.*109(1): 61-70.

Badri, J., Yepuri, V., Anuradha., G., Sivaramakrishnan and Siddiq, E.A. 2014. Development of microsatellite markers in sesame (*Sesamum indicum* L.). *TurkishJournal of Agriculture and Forestry*. 38: 603-614.

Bal, L.M., Satpute, G.K., Srivastava, A.K and Mishra, S.P. 2021. Molecular Diversity Assessment in Selected Accessions of White Seeded Sesame (*Sesamum indicum* L.) using SSR Markers. *Journal of Scientific & Industrial Research*. 80(04): 315-321.

Bamrotiya, M.M., Patel, J.B., Malav, A., Chetariya, C.P., Ahir, D and Kadiyara, J. 2016. Genetic Variability, Character Association and Path Analysis in Sesame (*Sesamum indicum* L.). *International Journal of Agriculture Sciences*. 8(54): 2912-2916.

Banerjee, P.P and Kole, P.C. 2009. Analysis of Genetic Architecture for Some Physiological Characters in Sesame (*Sesamum indicum* L.). *Euphytica.* 168: 11-22

Barela, A., Shrivastava, M.K., Mohare, S., Rahangdale, S., Jawarkar, S., Amrate, P.K and Singh, Y. 2022. Morphological characterization and recognition of new traits of soybean [*Glycine max* (L.) Merrill]. *International Journal of Environment and Climate Change*. 12(12): 1497-1504.

Barik, J., Kumar, V., Lenka, S.K and Panda, D. 2019. Genetic potentiality of lowland indigenous indica rice (*Oryza sativa* L.) landraces to anaerobic germination potential. *International Journal of Current Microbiology and Applied Sciences*. 8(12): 2527-2538.

Bharathi, D., Thirumalarao, V., Chandra, M.Y., Bhadru, D and Venkanna, V. 2014. Genetic variability studies in sesame (*Sesamum indicum* L.). *International Journal of Applied Biology and Pharmaceutical Technology*. 5(4): 31-33.

Bhat KV, Babrekar PP, Lakhanpaul S. Study of genetic diversity in Indian and exotic sesame (S*esamum indicum* L.) germplasm using random amplified polymorphic DNA (RAPD) markers. Euphytica. 1999. 110(1): 21-33.

Bhat, K. Kumari, R. Pathak, N. Rai, A. Value addition in sesame: A perspective on bioactive components for enhancing utility and profitability. *Pharmacognosy Reviews*. 2014. 8:147–155.

Bhatt, G.M. 1973. Comparison of various methods of selecting parents for hybridization in common bread wheat (*Triticum aestivum* L.). *Australian journal of agricultural research*. 24(4): 457-464.

Bhattacharjee, M., Iqbal, A., Singha, S., Nath, D., Prakash, S.H and Dasgupta, T. 2019. Genetic diversity in *Sesamum indicum* L. *Bangladesh Journal of Botany*. 48(3).497-506.

Bhattacharjee, M., Prakash, S.H., Roy, S., Begum, T and Dasgupta, T. 2018. DNA fingerprinting of CUMS 17 (Suprava): a newly developed variety of sesame. *International Journal of Pure Applied Biosciences*. 6(5): 161-166.

Bhoot, H.V., Sharma, L.K., Kulkarni, G.U., Ravat, U and Rathva, S. 2019. Characterization of sesame genotypes through morphological characters. *Journal of Pharmacognosy and Phytochemistry*. 8(3): 3132-3138.

Bhuiyan, M.S.H., Malek, M.A., Sarkar, M.A., Islam, M and Akram, M.W. 2019. Genetic variance and performance of sesame mutants for yield contributing characters. *Malaysian journal of sustainable agriculture*. 3(2): 27-30.

Bindu, M.R., Sushamakumari, P., Indira, M., Vilasini, T.N., Seeja, S and Yohannan, A.A. 2014. Genetic variability, heritability and genetic advance for yield and its components in sesame (*Sesamum indicum* L.). *International Journal of Plant Sciences.* 9(1): 167-169.

Bisen, R. Tripathi, A., Ravindra, P.A., Paroha, S., Sahu, R and Ranganatha. A.R.G. 2013. Study on genetic divergence in sesame (*Sesamum indicum* L.) germplasm based on morphological and quality traits. *The Bio-Scan: An International Quarterly Journal of Life Sciences.* 8(4): 1387-1391.

Burton, G.W. 1952. Quantitative inheritance in grasses. *Proceedings of the 6th International Grassland Congress*. 277-283.

Dar, A.A., Mudigunda, S., Mittal, P.K and Arumugam, N. 2017. Comparative assessment of genetic diversity in *Sesamum indicum* using RAPD and SSR markers. *3 Biotech.* 7*:* 10.

Divya, K., Rani, T.S., Babu, T.K and Padmaja, D. 2018. Assessment of genetic variability, heritability and genetic gain in advanced mutant breeding lines of sesame (*Sesamum indicum* L.). *International Journal of Current Microbiology and Applied Sciences*.7(6): 1565-1574.

Dossa, K. Wei, X. Zhang, Y. Fonceka, D. Yang, W. Diouf, D. Liao, B. Cisse, N. Zhang, X. Analysis of Genetic Diversity and Population Structure of Sesame Accessions from Africa and Asia as Major Centers of Its Cultivation. *Genes* .2016. 7:14.

Durga, G.V., Babu, D.R., Lal Ahamed, M and Rao, V.S. 2014. Genetic Variability, Heritability and Genetic Advance for Seed Yield and its Components in Sesame (*Sesamum Indicum* L.).

Ercan, A.G., Taskin, K.M., Turgut, K., Bilgen, M and Firat, M.Z. 2002. Characterization of Turkish sesame (*Sesamum indicum* L.) landraces using agronomic and morphologic descriptors. *Ziraat Fakultesi Dergisi Akdeniz universitesi*. 15(2): 45-52.

Evangelia, S., Georgios, L., Parthena, K., Ioannis, G. , Irini ,B., Eleni,T.,Stiliani, T., Apostolos, K., Ioannis, G and Panagiotis, M. 2021. Characterization of the genetic diversity present in a diverse sesame landrace collection based on phenotypic traits and EST-SSR markers coupled with an HRM Analysis. *Plants*. (10): 656.

Falusi, O.A., Yahaya, S.A., Gado, A.A., Daudu, O.A.Y., Akinbo, O.A and Teixeira da Silva, J.A. 2015. Morphological evaluation of selected sesame (*Sesamum indicum* L.) genotypes from five states in northern Nigeria. *African Journal of Agricultural Research.* 10(37): 3657-3661.

Fiseha, B., Yemane, T and Fetien, A. 2015. Assessing inter-relationship of sesame genotypes and their traits using cluster analysis and principal component analysis methods. *International Journal of Plant Breeding and Genetics.* 9(4): 228-237.

Fisher, R.A and Yates, F. 1963. Statistical Tables for Biological, Agricultural and Medical Research, Sixth edition, Oliver and Boyd, Edinburgh.

Frary A, Tekin P, Celik I, Furat S, Uzun B, Doganlar S. 2015. Morphological and Molecular Diversity in Turkish Sesame Germplasm and Core Set Selection. *Crop Science*. 55:702-711.

Furat, S and Uzun, B. 2010. The Use of Agro-morphological Characters for the Assessment of Genetic Diversity in Sesame (*Sesamum indicum* L). *Plant Omics*. 3(3): 85-91.

Gao YL, Zhu RS, Liu CY, Li WF, Jiang HW, Li CD, Chen QS. 2009. Constructing molecular identity for soybean varieties from Heilongjiang Province, China. *Acta Agronomica Sinica*. 35:211–8.

Gidey, Y.T., Kebede, S.A and Gashawbeza, G.T. 2013. Assessment of genetic variability, genetic advance, correlation and path analysis for morphological traits in sesame genotypes. *International Journal of Plant Breeding and Genetics*. 7(1): 21-34.

Gilbert, N.E.G. 1958. Diallel cross in plant breeding. *Heredity*. 12(4): 477-492.

Gogoi, L.R., Singh, S.K and Sarma, R.N. 2018. Assessment of genetic diversity in indigenous sesame genotypes. *International Journal of Current Microbiology and Applied Sciences*. **7**(6): 1509- 1520.

Haibru, G., Dash, M., Pradhan, B., Lenka, D and Tripathy, S.K. 2018. Genetic parameters of variability and character association studies for yield and some capsule shattering traits in sesame germplasm. *Journal of Pharmacognosy and Phytochemistry*. 7(5): 585-590.

Hika, G., Geleta, N and Jaleta, Z. 2015. Genetic variability, heritability and genetic advance for the phenotypic traits in sesame (*Sesamum indicum* L.) populationsfrom Ethiopia. *Science, Technology and Arts Research Journal*. 4(1): 20-26.

http: www.the-qrcode-generator.com

http: www.Indiastat.com. 2021-22.

Hwang, T. Y., Sayama, T. A., Takahashi, M. A., Takada, Y. O., Nakamoto, Y. U and Funatsuki, H. L., et al. 2009. High density integrated linkage map based on SSR markers in soybean. DNA Research. 16(4): 213-225.

Imran, M., Dash, M., Das, T.R and Kabi, M. 2018. Analysis of induced genetic variability for morphological and floral characters with male sterility in sesame (*Sesamum indicum* L.). *Electronic Journal of Plant Breeding*. 9(3): 801-807.

Iqbal, A., Akhtar, R., Begum, T and Dasgupta, T. 2016. Genetic estimates and diversity study in Sesame (*Sesamum indicum* L.). *IOSR Journal of Agriculture and Veterinary Science*. 9(8).1-5.

Islam, M. N., M. R. Molla and L. Rahman. (2013). Genetic characterization of sesame varieties through RAPD fingerprinting technique in Bangladesh.

Jamali, S.H., Cockram, J and Hickey, L.T. 2019. Insights into deployment of DNA markers in plant variety protection and registration. *Theoretical and Applied Genetics*. 132: 1911-1929.

Jamir, M., Changkija, S and Chaturvedi, H.P. 2020. Genetic Diversity in Sesame (*Sesamum indicum* L.) Landraces of North East India. *Indian Research Journal of Genetics and Biotechnology*. 12(02): 88-92.

Johnson, H.W., Robinson, H.F and Comstock, R.E. 1955. Estimates of genetic and environmental variability in soyabean. *Agronomy Journal.* 47: 314-318.

Jordens, R. 2005. Progress of plant variety protection based on the International Convention for the Protection of New Varieties of Plants (UPOV Convention). *World Patent Information*. 27(3): 232-243.

Joshi, M.A., Aggarwal, D and Sanyal, A. 2018. Cultivar identification and diversity analysis based on morphological descriptors and image analysis in chickpea (*Cicer arietinum* L.). *Legume Research-An International Journal*. 41(5): 647-655.

Kabi, M., Bhabendra, B., Manasi, D and Tripathy, S.K. 2020. Study of genetic diversity based on quantitative traits in sesame. *Journal of pharmaceutical innovation*. 9(7): 186-190.

Kadvani, G., Patel, J.A., Patel, J.R., Prajapati, K.P and Patel, P.J. 2020. Estimation of genetic variability, heritability and genetic advance for seed yield and its attributes in sesame (*Sesamum indicum* L.). *International Journal of Bio-resource and Stress Management*. *11*(3): 219-224.

Kalaiyarasi, R., Lokeshkumar, K., Mohanraj, M., Priyadharshini, A and Rajasekar, R. 2019. Genetic variability parameters for yield and yield related traits in sesame (*Sesamum indicum* L.). *International Journal of Current Microbiology and Applied Sciences*. 8(8): 819-825.

Kant, C., Mishra, S.P., Mani, N and Kumar, N. 2021. Assessment of genetic variability & some biochemical traits in Sesame (*Sesamum Indicum* L.) genotypes. *Journal of Pharmacognosy and Phyto-chemistry*. 10(5): 252-256.

Kaur, G. Joshi, A. Jain, D. SSR-Marker assisted evaluation of Genetic Diversity in Mungbean (*Vigna radiata* L.) genotypes. *Brazilian. Archives of Biology and Technology*. 2018. 61.

Kehie, T., Shah, P., Chaturvedi, H.P and Singh, A.P. 2020. Variability, correlation and path analysis studies in sesame (*Sesamum indicum* L.) genotypes under foothill condition of Nagaland. *International Journal of Current Microbiology and Applied Sciences.* 9(5): 2917-2926.

Khan, M.A., Mirza, M.Y., Akmal, M., Ali, N and Khan, I. 2007. Genetic parameters and their implications for yield improvement in sesame. *Sarhad Journal of Agriculture*. 23(3): 623.

Kiranmayi, S.L., Roja, V., Sivaraj, N and Sivaramakrishnan, S. 2016. Genetic diversity analysis in sesame(*Sesamum indicum*) using morphological, biochemical and molecular techniques. *International Journal of Applied Biology and Pharmaceutical Technology*. 7: 95-110.

Kiruthika, S., Narayanan, S.L., Parameswari, C., Mini, M.L and Arunachalam, P. 2018. Genetic variability studies for yield and yield components in sesame (*Sesamum indicum* L.). *Electronic journal of plant breeding*. 9(4): 1529-1537.

Kola, V.S.R., Yepuri, V., Surapaneni, M., Badri, J., Vemireddy, L.R., Ghanta, A and Siddiq, E.A. 2012. Genetic diversity and DNA fingerprinting in sesame (*Sesamum indicum* L.) cultivars of ANGRAU. *Asian and Australasian journal of plant science and biotechnology.* 16: 98-101.

Kumar, V and Sharma, S.N. 2011. Comparative potential of phenotypic, ISSR and SSR markers for characterization of sesame (*Sesamum indicum* L.) varieties from India. *Journal of Crop Science and Biotechnology*. 14(3): 163-171.

Kumar, V., Sinha, S., Sinha, S., Singh, R.S and Singh, S.N. 2022. Assessment of genetic variability, correlation and path analysis in sesame (*Sesamum indicum* L.). *Electronic journal of plant breeding*. 13(1): 208-215.

Kumari, M. 2017. Molecular characterization of released varieties of sesame (*Sesamumindicum* L.) using SSR markers. *M.Sc (Ag) Thesis*, Indian Agricultural Research Institute, New Delhi.

Kumari, M., Choudhary, B.R., Bhardwaj, R and Godara, A. 2020. Morphological Characterization in Sesame (*Sesamum indicum* L.) for Seed Yield and its Component Traits. *International Journal of Current Microbiology and Applied Sciences.* 11: 550-555.

Kumari, P., Shah, P and Chaturvedi, H.P. 2023. Studies on Yield Attributes in Relation to Genetic Parameters in Sesame (*Sesamum indicum* L.) Genotypes.

Kwon YS, Lee JM, Yi GB, Kim KM, Soh EH, Bae KM. Use of SSR markers to complement tests of distinctiveness, uniformity, and stability (DUS) of pepper (*Capsicum annuum* L.) varieties. *Molecules and Cells*. 2005.19(3):428-435.

Laurentin, H. Karlovsky, P. AFLP fingerprinting of sesame (*Sesamum indicum* L.) cultivars: Identification, genetic relationship and comparison of AFLP informativeness parameters. *Genetic Resources and Crop Evolution*. 2007. 54: 1437–1446.

Lush, J.L. 1940. Intra-series correlation and regression of offspring in rams as method of estimating heritability of characters. *Proceedings of American Society of Animal Product.* 33: 292-301.

Lush,J.L.1949. Heritability of quantitative characters in farm animals. *Heritability of quantitative characters in farm animals.*

Mahalanobis, P.C. 1936. On the generalized distance in statistics. *Proceedings of National Institute of Sciences*. India. 12: 49-55.

Mahesh, S.2014. Characterization of sesame genotypes through morphological, biochemical and molecular (RAPD) markers. *M.Sc. (Ag.) thesis.* Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani.

Mahmoud, M.W., Abo-Elezz, A.A and Hassan, T.H.A. 2015. Genetic variability, heritability and correlation coefficients of yield and its components in sesame. *Egyptian Journal of Plant Breeding*. 203(3826): 1-16.

Manjeet, P.K., Sheoran, R.K., Nain, M and Avtar, R. 2020. Evaluation of sesame (*Sesamum indicum* L.) genotypes for genetic variability based on different traits under rainfed conditions. *Electronic Journal of Plant Breeding*. 11(01): 54-59.

Mathur, S. 2022. Morphological Characteristics of Sixteen Sesamum Varieties for Distinctness, Uniformity and Stability (DUS) Test. *International Association of Biologicals and Computational Digest*. 1(2): 247-253.

Mawcha, K.T., Gebru, M.M., Assefa, M.K., Mesfin, M and Gebre, G.G. 2020. Morphological Characterization and Genetic Diversity of Sesame (L.) Varieties Cultivated in Ethiopia. *The Open Agriculture Journal*.14(1).

Menzir, A. 2012. Phenotypic variability, divergence analysis and heritability of characters in sesame (*Sesamum indicum* L.) genotypes. *Nature and Science*. 10(10): 117-126.

Mohan, Y.C. 2014. Variability and genetic divergence in sesame (*Sesamum indicum* L.). *International Journal of Applied Biology Pharmaceutical Technology.* 5(3): 222-225.

Mohanty, T.A., Singh, U.M., Singh, S.K., Singh, D and Kushwaha, N. 2020. Assessment of Genetic Diversity in Sesame (*Sesamum indicum* L.) Based on Agro-Morphological Traits. *Current Journal of Applied Science and Technology.* 39(25): 100-107.

Morris, J.B. 2009. Characterization of sesame (*Sesamum indicum* L.) germplasm regenerated in Georgia, USA. *Genetic Resources and Crop Evolution*. *56*: 925-936.

Mourad, K.A., El-Khalek, A., Rasha, Y.S and Naguib, N.A. 2019. Determination of genetic diversity in sesame. *Zagazig Journal of Agricultural Research*. 46(2): 279-293.

Mukhthambica, K., Bisen, R and Ramya, K.T. 2023. Genetic diversity analysis in sesame (*Sesamum indicum* L.). *Electronic Journal of Plant Breeding*. 14(1): 303-313.

Nandakumar, N. Singh, A.; Sharma, R. Mohapatra, T. Prabhu, K. Zaman, F. Molecular fingerprinting of hybrids and assessment of genetic purity of hybrid seeds in rice using microsatellite markers. *Euphytica*. 2004. 136: 257–264.

Narayanan, R and Murugan, S. 2013. Genetic divergence and stability analysis in sesame (*Sesamum indicum*). *International journal of advanced Research*. 2(11): 16-19.

Noli, E., Teriaca, M.S., Sanguineti, M.C and Conti, S. 2008. Utilization of SSR and AFLP markers for the assessment of distinctness in durum wheat. Molecular Breeding. 22: 301-313.

Ozcinar AB, Sogut T. Analysis of sesame (*Sesamum indicum* L.) accessions collected from different parts of turkey based on qualitative and

quantitative traits. *Journal of Crop Breeding Genetics*. 2017. 3(1):45-51.

Palakshappa, M.G., Banu, H., Parmeshwarappa, S.G., Bisen, R., Nagappa, H and Holeyannavar, P. 2020. DUS testing of sesame (*Sesamum indicum* L.) Accessions using morphological descriptors and evaluation for foliar diseases of sesame. *International Journal of Current Microbiology and Applied Sciences*. 9(1): 1837-1852.

Pandey, S.K., Das, A., Rai, P and Dasgupta, T. 2015. Morphological and genetic diversity assessment of sesame (*Sesamum indicum* L.) accessions differing in origin. *Physiology and molecular biology of plants*. 21: 519-529.

Panse, V.G and Sukhatme, P.V. 1961. Statistical Methods for agricultural workers. 2nd edition, Indian Council of Agricultural Research, New Delhi. 361.

Parameshwarappa SG, Palakshappa MG, Salimath PM, Parameshwarappa KG. Evaluation and characterization of germplasm accessions of sesame (*Sesamum indicum* L.). Karnataka Journal of Agricultural Sciences. 2008. 22(5):1084-1086.

Parameshwarappa, S. G., Palakshappa, M. G and Gayatree, G. S. 2012. Studies on genetic divergence for yield and yield attributing traits in sesame (*Sesamum indicum* L.) germplasm. *International Journal Agricultural Sciences.* 8(2): 441-444.

Parsaeian, M. Mirlohi, A. Saeidi, G. Study of genetic variation in sesame (*Sesamum indicum* L.) using agro-morphological traits and ISSR markers. *Russian Journal of Genetics*. 2011. 47: 314–321.

Patel, N.B., Acharya, R.R., Macwana, S.M., Halladakeri, P., Parihar, A and Parmar, D.J. 2022. Genetic variability, correlation and path analysis for seed yield and yield contributing traits in sesame (*Sesamum indicum* L.) germplasm. *The Pharma Innovation Journal*. 12(2): 3781-3786.

Patil, M and Lokesha, R. 2018. Estimation of genetic variability, heritability, genetic advance, correlations and path analysis in advanced mutant

breeding lines of sesame (*Sesamum indicum* L.). *Journal of Pharmacognosy & Natural Products.* 4: 151.

Patil, M.K., Lokesha, R and Diwan, J.R., 2018. Genetic divergence of advanced mutant breeding lines in sesame (*Sesamum indicum* L.) assessed through D^2 statistics. *International Journal of Current Microbiology and Applied Science*. 6(9):3133-3139.

Pavani, K., Lal Ahamed, M., Ramana, J.V and Sirisha, A.B.M., 2020. Studies on genetic variability parameters in sesame (*Sesamum indicum* L.). *International Journal of Chemical Studies*. 8(4): 101-104.

Perrier, X. 2006. DARwin software. *http://darwin. cirad.fr/darwin*.

Porebski, S.L., Bailey, L.G and Baum, B.R. 1997. Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Molecular Biology Reporter.* 15(1): 8-15.

Prithviraj, S.K and Parameshwarappa, S.G. 2017. Genetic variability studies for quantitative traits in germplasm collections of sesame (*Sesamum indicum* L.). *Journal of Farm Sciences*. 30(2): 149-152.

Protection of Plant Varieties and Farmers' Rights Authority (PPV & FRA). 2001. Government of India. Guidelines for the conduct of test for distinctiveness, uniformity and stability on Sesame (*Sesamum indicum* L.).

Rajitha, D., Srikanth, T., Padmaja, D and Babu, T.K. 2018. Genetic divergence anal genetic divergence analysis of sesame genotypes ysis of sesame genotypes (*Sesamum indicum* L.).

Ramprasad, E., Senthilvel, S., Jatothu, J.L., Yamini, K.N and Dangi, K.S. 2019. Analysis of genetic diversity in sesame (*Sesamum indicum* L.) advanced breeding lines and varieties collected from major breeding centers in India.

Ramprasad, E.; Senthilvel, S.; Jawahar, J.; Yamini, K. N., Dangi K. S.; Ranganatha, A.R.G and Varaprasad, K. S. 2017. An insight into

morphological and molecular diversity in indian sesame cultivars, *Indian Journal of Genetics*. 77(2): 271-277.

Rao, C.R. 1952. Advanced statistical methods in biometrical research. John Wiley and Sons INC., New York. 357-363.

Roy, B., Pal, A.K and Basu, A.K. 2022. The estimation of genetic variability and genetic divergence of some advance lines of sesame based on morphological traits. *Plant Science Today*. 9(2): 281-287.

Sabiel, S.A.I., Ismail, M.I., Abdalla, E.A. and Osman, A.A. 2015. Genetic variation in sesame genotypes (*Sesamum indicum* L.) grown in the semi-arid zone of the Sudan. *SABRAO Journal of Breeding and Genetics*. 47(3): 214-220.

Saha, S., Barik, S and Kothari, S.K. 2023. Genetic variability for yield and yield attributing traits in sesame (*Sesamum indicum* L.) genotypes under soil salinity stress. *Journal of Pharmacognosy and Phyto-chemistry*. 12(1): 101-104.

Sahu, D., Mishra, T.K and Pradhan, B. 2022. Genetic variability, heritability and genetic advance in different genotypes of sesame (*Sesamum indicum* L.). *The Pharma Innovation Journal*. 11(11): 1799-1801.

Salazar B, Laurentin H, Davila M, Castillo MA. Reliability of the RAPD technique for germplasm analysis of sesame (*Sesamum indicum* L.) from Venezuela. *Interciencia.* 2006. 31(6): 456-60.

Sarita, K. Pandey, Arna Das and Tapash Dasgupta. 2013. Genetics of seed coat color in sesame (*Sesamum indicum* L.). *African Journal of Biotechnology*. 12 (42): 6061-6067

Sasipriya, S., Parimala, K., Balram, M and Eswari, K.B. 2022. Variability and character association in sesame (*Sesamum indicum* L.). *The Pharma Innovation Journal*. 11(1): 299-302.

Sasipriya, S., Parimala, K., Balram, M and Eswari, K.B. 2023. Assessment of Genetic Diversity in Sesame (*Sesamum indicum* L.) for Yield and its Components. *International Journal of Bio-resource and Stress Management*. *14*: 443-449.

Sasipriya, S.; Balram, M.; Parimala, K.; Eswari, K. B. (2020). Assessment of molecular divergence in sesame (*Sesamum indicum* L.) genotypes using microsatellite (SSR) markers, *International Journal of Ecology and Environmental Sciences*. 2(4): 182-187.

Savaliya, J. 2021.Characterization of black sesame (*Sesamum indicum* L.) genotypes through plant morphological characters and chemical test. *M.Sc. (Ag.) thesis*. Junagadh Agricultural University, Junagadh.

Saxena, K. and Bisen, R. 2017. Genetic variability, heritability and genetic advance for the phenotypic traits in sesame (*Sesamum indicum* L.). *International Journal of Pure and Applied Bioscience*. 5(2): 1126-1131.

Sehr, E.M. Okello-Anyanga, W. Hasel-Hohl, K. Burg, A. Gaubitzer, S. Rubaihayo, P.R. Okori, P. Vollmann, J. Gibson, P. Fluch, S. Assessment of genetic diversity amongst Ugandan sesame (*Sesamum indicum* L.) landraces based on agromorphological traits and genetic markers. *Journal of Crop Science Biotechnology*. 2016. 19: 117–124.

Singh, A., Bisen, R. and Tiwari, A. 2018. Genetic variability and character association in sesame (Sesamum indicum L.) genotypes. *International Journal of Current Microbiology and Applied Sciences*. 7(11): 2407-2415.

Singh, B., Bisen, R and Tiwari, A. 2017. DUS testing of sesame (*Sesamum indicum* L.) varieties using morphological descriptors. *Bulletin of Environment, Pharmacology and Life Sciences*. 6(1): 05-12.

Singh, K.M., Kumar, D.B., Kumar, D.S and Manorama. 2015. Assessment of genetic diversity among Indian sesame (*Sesamum indicum* L.) accessions using RAPD, ISSR and SSR markers. *Research Journal of Biotechnology*. 10(8): 35-47.

Singh, R.K and Chaudhary, B.D. 1977. *Biometrical Methods in Quantitative Genetic Analysis.* Kalyani Publishers, New Delhi. 215-218.

Singh, U.K., Kumar, N., Mandal, R.K., Kumar, M and Kumar, R. 2022. Analysis of genetic variability, character association and path

analysis in sesame (*Sesamum indicum* L.). *The Pharma Innovation Journal*. 11(7): 2215-2221.

Sirisha, A.B.M., Banu, S.H and Saritha, R. 2020. Assessment of genetic variability, heritability in sesame (*Sesamum indicum* L.*). International Journal of Chemical Studies*. 8(4): 2262-2264.

Sivabharathi, R.C., Muthuswamy, A., Anandhi, K and Karthiba, L. 2022. Characterization of soybean [*Glycine max* (L.) Merrill] genotypes using DUS descriptors. *Electronic Journal of Plant Breeding*. 13(2): 455-464.

Sivasubramanian, S and Madhavanmenon. 1973. Heterosis and inbreeding depression in rice. *Madras Agricultural Journal*. 60: 1139-1144.

Soundharya, B., Hemalatha, V., Rani, T.S and Chary, D.S. 2016. Genetic variability, heritability and genetic advance for the phenotypic traits in sesame (*Sesamum indicum*. L) genotypes.

Soundharya, B., Hemalatha, V., Shobha Rani, T and Edukondalu, B. 2017. Genetic divergence studies in sesame (*Sesamum indicum* L.) genotypes. *International Journal of Current Microbiology and Applied Sciences.* 6(9): 2615-2619.

Srikanth, K and Ghodke, M.K. 2022. Genetic diversity studies in sesame (*Sesamum indicum* L.) genotypes.

Suganthi, S. 2017. Genetic divergence studies in sesame (*Sesamum indicum* L.). In *UGC SAP National Seminar on Emerging Trends In Crop Improvement*. 195.

Suhasini, K.S. 2006. Characterization of sesame genotypes through morphological, chemical and RAPD markers. *M Sc.(Agri.) Thesis, Dharwad University of Agricultural Sciences, Dharwad, India*.

Sultana, S., Mahmud, F and Rahim, M.A., 2019. Genetic variability studies for selection of elite germplasm in sesame (*Sesamum indicum* L.). *Agronomski glasnik: Glasilo Hrvatskog agronomskog društva*. 81(2): 87-104.

Sumathi, P and Muralidharan, V. 2011. Analysis of genetic variability, association and path analysis in the hybrids of sesame (*Sesamum indicum* L). *Tropical Agricultural Research and Extension*. 13(3).

Surapaneni, M., Yepuri, V., Vemireddy, L.R., Ghanta, A and Siddiq, E.A. 2014. Development and characterization of microsatellite markers in Indian sesame (*Sesamum indicum* L.). *Molecular Breeding.* 34: 1185–1200.

Swapna, B., Thippani, S., Padmaja, D and Nayak, S.S. 2023. Genetic Diversity for Yield and Yield Contributing Characters in Sesame (*Sesamum indicum* L.). *International Journal of Environment and Climate Change*. 13(8): 1717-1725.

Swathy, V., Premnath, A., Viswanathan, P.L, Raveendran, M and Manonmani, S.2018. Determination of genetic divergence based on morphological traits in sesame (*Sesamum indicum* L.). *Electronic Journal of Plant Breeding*. 9(2): 747-752.

Takele, F and Abera, G. 2023. Variability Study in Ethiopian Sesame (*Sesamum indicum* L.) Genotypes at Western Oromia. *International Journal of Precision Farming*. 1(1): 1-7.

Tanwar, A and Bisen, R. 2018. Genetic diversity analysis in sesame (*Sesamum indicum* L.) germplasm based on morphological and quality traits. *ElectronicJournal of Plant Breeding*. 9(1): 9-17.

Teklu, D. H., Kebede, S. A and Gebremichael. 2014, Assessment of genetic variability, genetic advance, correlation and path analysis for morphological traits in sesame genotypes. *Asian Journal of Agricultural Research*.1-6.

Tesfaye, T., Tesfaye, K., Keneni, G and Alemu, T. 2021. Morphological characteristics and genetic diversity of Ethiopian sesame genotypes. *African Crop Science Journal*. 29(1): 59-76.

Thomson, M.J. Septiningsih, E.M. Suwardjo, F. Santoso, T.J. Silitonga, T.S. McCouch, S.R. Genetic diversity analysis of traditional and improved Indonesian rice (*Oryza sativa* L.) germplasm using microsatellite markers. *Theoretical Applied Genetics* .2007. 114: 559–568.

Tripathi, A., Rajani, S., Ravindra, P., Ahirwal., Seema, P., Roshni, S and Ranganatha, A. R.G. 2014. Study on genetic divergence in sesame (*sesamum indicum* L.) Germplasm based on morphological and quality traits. *The bioscan*. 8(4): 1387-1391.

Tripathi, A.N.J.A.Y., Bisen, R.A.J.A.N.I., Ahirwal, R.P., Paroha, S., Sahu, R and Ranganatha, A.R.G. 2013. Study on genetic divergence in sesame (*Sesamum indicum* L.) germplasm based on morphological and quality traits. *The bioscan*. 8(4): 1387-1391.

Tripathy, S.K., Mishra, D.R., Panda, S., Senapati, N., Nayak, P.K., Dash, G.B., Mohanty, S.K., Mohanty, M.R., Jena, M and Pradhan, K., 2016. Assessment of genetic variability in sesamum (*Sesamum indicum* L.). *Asian Journal of Science and Technology*. 7(2): 2482-2485.

Uikey, K.N., Deshmukh, D.T., Vaidya, E.R., Mohurle, N.A., Gomashe, S.S., Durge, D.V. and Walke, R.D. 2022. Genetic divergence study among the germplasm of sesame (*Sesamum indicum* L.).

Ukaan, S and Ogbonna, P. 2012. Genetic variability and character association in sesame (*Sesamum indicum* L.) accessions. *International Journal of Plant Breeding*. 6(2): 139-143.

Ukani, J.2018. Characterization of sesame genotypes through plant morphology, chemical, chemical tests and molecular markers. *Ph.D. (Ag.)* thesis. Junagadh Agricultural University, Junagadh.

Umamaheswari, S., Suganthi, S., Sathiskumar, P and Kamaraj, A. 2019. Genetic variability, correlation and path analysis in sesame (*Sesamum indicum* L.). *Plant Archives*. 19(2):4543-4548.

Valarmathi, G., Surendran, C., Vanniarajan, C., Kumar, M and Saravanan, N.A. 2003. Morphological and biochemical characterization of sesame (*Sesamum indicum* L. and *Sesamum mulayanum* L). *Sesame and Safflower Newsletter*. 18: 42-46.

Vamshi, P., RAMYA, K., PRASAD, M.S., RAMANA, J. and KUMHAR, S. 2021. Genetic variability studies for yield and yield attributes in breeding lines of sesame (*Sesamum indicum* L.). *Indian Society of Oilseeds Research*. 38(3): 240-243.

Vanishree, S.G., Banu, H., Palakshappa, M.G and Holeyannavar, P. 2022. Morphological characterization of sesame (*Sesamum indicum* L.) germplasm accessions with DUS.

Wang, L, Yu, S., Tong, C., Zhao, Y., Liu, Y., Song, C. (2014). Genome sequencing of the high oil crop sesame provides insight into oil biosynthesis. *Genome Biology*. 15: 39.

Wei, X., Gong, H., Yu, J., Liu, P.,Wang, L., Zhang, Y. (2017). Sesame FG: An integrated database for the functional genomics of sesame. *Scientific Reports*. 7 (1): 1–10.

Wu, K. Yang, M. Liu, H. Tao, Y. Mei, J. Zhao, Y. Genetic analysis and molecular characterization of Chinese sesame (*Sesamum indicum* L.) cultivars using Insertion-Deletion (InDel) and Simple Sequence Repeat (SSR) markers. BMC Genetics. 2014. 15: 35.

Yadav, B.P., Thiruvengadam, V., Sasikala, R and Rajendran, L. 2022. Analysis of genetic diversity in sesame (*Sesamum indicum* L.) germplasm for yield and its attributing traits. *Electronic Journal of Plant Breeding*. 13(3):927-931.

Yepuri, V., Surapaneni, M., Kola, V., Vemireddy, L.R., Jyothi, B and Dineshkumar,V. 2013. Assessment of genetic diversity in sesame (*Sesamum indicum* L.) genotypes using EST-derived SSR markers. *Journal of Crop Sciences and Biotechnology*. 16: 93–103.

Zhang, H., Wei, L., Miao, H., Zhang, T and Wang, C. 2012. Development and validation of genic-SSR markers in sesame by RNA-seq. *BMC Genomics.* 13: 316.

Appendices

APPENDIX - A

LIST OF CHEMICALS

1. Agarose (Himedia)
2. Bromophenol blue (Qualigens)
3. 6X loading dye (Takara)
4. Chloroform (Himedia)
5. Cetyl Trymethyl Ammonium bromide (CTAB)
6. dNTP's (Deoxy Nucleoside Triphosphate) (GeNei ^{TM})
7. Ethylene Diamine Tetra Acetic Acid (EDTA) (Himedia)
8. Ethidium bromide (10 mg/ml) (Himedia)
9. Ethyl alcohol (Himedia)
10. Icecold isopropanol (Himedia)
11. Isoamyl alcohol (Himedia)
12. 2- Mercaptoethanol GR (Himedia)
13. NaOH pellets (Sodium Hydroxide) (Himedia)
14. NaCl (Sodium Chloride) (Himedia)
15. Phenol (Himedia)
16. Poly vinyl pyrrolidine (Merck Lifesciences)
17. Proteinase K (Macherey-Nagel)
18. RNase H (Macherey-Nagel)
19. Taq polymerase (GeNei ^{TM})
20. 100bp ladder (Thermo Scientific)

APPENDIX - B

BUFFERS AND STOCK SOLUTIONS

CTAB buffer 100ml

2.0 g CTAB (Hexadecetyltrimethyl- ammonium bromide)

10.0 ml 1 M Tris pH 8.0

4.0 ml 0.5 M EDTA pH 8.0 (Ethylenediamine tetra acetic acid)

28.0 ml 5 M NaCl

40.0 ml H_20

1g PVP 40 (Polyvinyl pyrrolidone- vinyl pyrrolidinehomopolymer, MW 40,000)Adjust pH to 8.0 by adding HCl and make up to 100 ml with distilled water

0.5 M Tris Buffer (pH 8.0)

Dissolved 60.55 g of Tris base in 400 ml of distilled water. Adjust pH to 8.0 by adding HCl. Adjust the volume to 500 ml with distilled water.

1M EDTA (Ethylenediamine tetra acetic acid)

Dissolved 186.1 grams of EDTA, free acid in about 200 ml of distilled water. Adjust the pH to 8.0 with NaOH and make up the volume to 500 ml with distilled water. Sterilized by autoclaving.

Ethidium Bromide

Stock 20mg / ml can be prepared by dissolving 1gm of ethidium bromide in 50ml of water.

Chloroform: Isoamyl alcohol (24:1)

24 ml of Chloroform is mixed with 1ml of Isoamyl alcohol (24:1) were mixedand stored at room temperature.

Phenol: Chloroform: Isoamyl alcohol (25:24:1)

Phenol: Chloroform: isoamyl alcohol (25:24:1) were mixed and stored at roomtemperature.

10 X TBE BUFFER, pH 8.3 (STOCK SOLUTION)

Tris base ($C_4H_{11}NO_3$)	-	108 g
Boric acid (H_3BO_3)	-	55 g
EDTA ($C_{10}H_{16}N_2O_8$)	-	9.3 g
Distilled water	-	1000 ml

Adjusted the pH to 8.3 and make up to the volume to 1 litwith distilled water. Sterilization is done by autoclaving.

TE buffer (pH 8.0)

10 mM TrisHCl

1 mM EDTA.

2 ml of 0.5 M TrisHCl pH 8.0 was mixed with 0.2 ml of 0.5 EDTA, make up tothe vol to 100 ml with sterile distilled water.

6X Gel loading buffer

0.25% (W/V)

Bromophenolblue40%

(W/V) sucrose in water

Dissolved 0.25g of Bromophenol blue was mixed with 40g of sucrose,make up the vol to 100ml with distilled water.

RNase preparation

RNase buffer

A. 1M Tris (pH 7.5)

B. 5M NaCl

Take 0.5ml of 1M Tris (final concentration 10mM) and 75 µl of 5M NaCl (final concentration 15mM) and make up the volume to 50 ml. Weighed 25 mg of ribonuclease H into a tube and add RNase buffer to a final volume of 5 ml (so final concentration 5mg / ml). Kept the tube in a boiling water bath for 10 min, cool and made aliquots of 1 ml in 1.5 ml Eppendorf tubes and stored at -20^0C.

APPENDIX - C
EQUIPMENTS USED

- Agarose gel electrophoresis system (A AHoefer, Richmond, CA 94804, USA)
- Autoclave (Equitron)
- Centrifuge (Eppendorf 5810 R and 5430 R)
- DNA Thermal Cycler (Agilent Sure Cycler 8800)
- Electronic balance (Mettler Toledo)
- Eppendorf tubes
- Freezer of -20ºC and -80ºC (Vest frost Solutions)
- Gel Documentation System (Syngene GBox F3)
- Glass hooks
- Incubator (Sanyo)
- Incubator shaker 37 ^{0}C (Labtop)
- Magnetic stirrer (Genei)
- Microcentrifuge tubes (Eppendorf)
- Microwave oven (Samsung)
- Nanodop (Thermo scientific 2000)
- pH meter (Eutech)
- Pipettes (Eppendorf)
- Power supply unit (Consort EV 245)
- UV- transilluminator (Labnet)
- Vortex mixer (Labnet)
- Water bath (Nuve NB 20)

DUS CHARACTERIZATION AND MOLECULAR DIVERSITY ANALYSIS IN SESAME (*Sesamum indicum* L.)

Department of Genetics and Plant Breeding, S.V. Agricultural college, Tirupati

Name of the Student: Amidala Manasa **I.D. No.** TAM/2021-027

Major advisor: Dr. D. Bharathi

ABSTRACT

The present investigation was conducted at Regional Agricultural Research Station, Tirupati during *Rabi*, 2022-23 to characterize sesame genotypes for DUS traits, to study the genetic parameters (variability, heritability and genetic advance) and genetic divergence and to develop varietal-specific DNA fingerprints of sesame using molecular markers.DUS characterization of 43 sesame genotypes using 20 DUS traits revealed the existence of substantial diversity among the characters and also appreciable differences were observed for the traits *viz.,* days to 50 % flowering, petal colour, petal hairiness, branching, branching pattern, stem hairiness, leaf lobes, leaf size, leaf serration of margin, capsule hairiness, capsule shape, capsule number per leaf axil, capsule arrangement, capsule length, days to maturity, seed colour, 1000-seed weight and oil content. These descriptors would aid in explicit the identity of genotypes. The analysis of variance carried out among 43 genotypes for 10 yield and yield attributes revealed significant differences among the genotypes for all the characters indicating the presence of considerable amount of genetic variability in the studied material. Higher estimates of PCV and GCV were exhibited by seed yield per plant indicating ample amount of variation among the genotypes for this trait. Thus, direct selection for this trait would be rewarding for improvement of yield. High heritability in conjunction with high genetic advance as per cent of mean was observed for number of primary branches per plant, number of capsules per plant and seed yield per plant indicating the predominance of additive gene action and direct selection would be effective for improvement of these traits. Genetic diversity studies indicated the existence of significant diversity among 43 sesame varieties and grouped them into seven clusters. Cluster II had maximum intra cluster distance while inter cluster distance was highest between cluster IV and VII followed by cluster III and IV, cluster IV and VI, cluster V and VII and cluster II and VII representing that genotypes belonging to these clusters were more divergent. Days to maturity contributed relatively maximum towards genetic divergence followed by oil content, number of primary branches per plant and days to 50 % flowering. The genotypes Paiyur-1 from cluster II, JCS-1020 from cluster VII, Nirmala from cluster IV, G.Til-4 from cluster III and Hima from cluster I could be selected as parents in future breeding programme as they expressed high *per se* performance for more number of traits, maximum inter cluster distance and complementarity for more number of traits of interest.Molecular profiling of 43 sesame genotypes revealed that out of 34 SSR markers used, 9 were polymorphic and generated alleles ranging from 2 to 4 with an average of 2.4 per locus. Polymorphism information content (PIC) values varied from 0.045 (SSR 212) to 0.549 (SEM 12-65) with an average of 0.279. The polymorphic percentage is 26%. DNA fingerprinting with 9 polymorphic SSR markers distinguished 17 genotypes whereas, by using both 12 DUS descriptors and alleles codes of nine polymorphic markers, differentiated all the 43 sesame varieties. The QR codes were generated for all the 43 genotypes using combination of DUS traits and allele codes. Therefore, both morphological and molecular diversity are important for the identification of varieties. In the present study, based on *per se* performance, morphological diversity using D^2 statistic and UPGMA cluster analysis, the crosses *viz.,* Paiyur-1 × Pratap, JCS-1020 × Nirmala, G.Til-4 × Pratap and Hima × Nirmala could be suggested for future breeding programme for development of high yielding sesame varieties.