**UGC Major Research Project**

**Project Report on**

# Genetic diversity among genotypes and molecular linkage map construction in sesame (Sesamum *indicum* L.)

**File No: F. 42-721/2013(SR)**
**Date: 21.03.2013**
**2013-2017**

**Submitted by Principal Investigator**
**Prof Tapash Dasgupta**
**Professor**
**Department of Genetics and plant Breeding**
**University of Calcutta**
**51/2 Hazra Road kolkta-700019**

Annexure –VIII

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002.

Annual/Final Report of the work done on the Major Research Project.
(Report to be submitted within 6 weeks after completion of each year)

1. Project report No. 1st /2nd /3rd/Final : **Final**

2. UGC Reference No. : **F. 42-721/2013(SR) dated 21.03.2013**

3. Period of report : **01.04.2013to 31.03.2017**

4. Title of research project : **Genetic diversity among genotypes and molecular linkage map construction in sesame (Sesamum *indicum* L.)**

5. (a) Name of the Principal Investigator : **Prof Tapash Dasgupta**

(b) Deptt. : **Genetics and Plant Breeding**

(c) University/College where work has progressed: **University of Calcutta**

6. Effective date of starting of the project : **01.04.2013**

7. Grant approved and expenditure incurred during the period of the report:

a. Total amount approved : **Rs. 12,55,980.00 (** Twelve lakh fifty five thousand Nine hundred Eighty only**)**

c. Total grant received : **Rs. 11,40,262.00 (** Eleven lakh Forty thousand Two hundred Sixty Two only**)**

d. Total expenditure : Rs. **12,94,721.00 (** Twelve lakh Ninty Four thousand Seven hundred Twenty One only**)**

e. Report of the work done : Separate sheet attached with brief objectives of the project and details of the performed work and achieved results

i. Publications, if any, resulting from the work : Four

**Research paper published:**

1. A. Iqbal and **T Dasgupta**, Genetic Estimates of Morphological Traits and Phenotypic Diversity in Core Collections of Sesame (Sesamum indicum L.) *Indian Agriculurist*, Vol.5, 2015, 61-69.

2. A. Iqbal., 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.
3. A. Iqbal, R. Das and **T. Dasgupta**. 2017 Association of seed iron and zinc content with seed yield and other traits in sesame *International Journal of Current Research*, 9, (07), 53847-53851.
4. Adil Iqbal, Pradip Kumar Pati Rumana Akhtar, Tamina Begum and **Tapash Dasgupta "Diversity in sesame accessions",** *International Journal of Agriculture, Environment and Biotechnology*, ***( in Press)***

ii. Has the progress been according to original plan of work and towards achieving the objective. if not, state reasons: : **See Annexure 1**

iii. Please indicate the difficulties, if any, experienced in implementing the project: **No difficulties worth mentioning were experienced in implementing the project**

iv. If project has not been completed, please indicate the approximate time by which it is likely to be completed. A summary of the work done for the period (Annual basis) may please be sent to the Commission on a separate sheet : **Not Applicable**

v. If the project has been completed, please enclose a summary of the findings of the study. One bound copy of the final report of work done may also be sent to University Grants Commission: **separate sheet has been enclosed as the summary of the finding of the studies. One bound copy of the final report of work done is also attached**

vi. Any other information which would help in evaluation of work done on the project. At the completion of the project, the first report should indicate the output, such as (a) Manpower trained (b) Ph. D. awarded (c) Publication of results (d) other impact, if any

The JRF appointed in the project has been registered at the University of Calcutta as Ph.D. student (Adil Iqbal, Registration No: **8482 Ph.D ( Ag. ) Proceed / 2014 dated. 17.12.2014**). the candidate has been submitted his thesis in July, 2017. Four publications have been already achieved from the project. Two manuscripts have been communicated for publication. Three more manuscripts is being prepared to be communicated for publication.

Dgupta 22/03/18

SIGNATURE OF THE PRINCIPAL INVESTIGATOR

Dr. Tapash Dasgupta
Ex- Professor in Genetics and Plant Breedin
Institute of Agricultural Science
University of Calcutta
35 B C Road Kolkata-700019

REGISTRAR 26/3

(Seal)

Registrar
University of Calcutta

Annexure – IX

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

# PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

| | |
|---|---|
| 1. Title of research project | : **Genetic diversity among genotypes and molecular linkage map construction in sesame ( *Sesamum indicum* L.)** |
| 2. NAME AND ADDRESS OF THE PRINCIPAL INVESTIGATOR: | **Prof Tapash Dasgupta**<br>**Dept. of Genetics and plant Breeding**<br>**51/2 Hazra Road Kolkata-700019** |
| 3. NAME AND ADDRESS OF THE INSTITUTION | : **Dept. of Genetics and plant Breeding, University of Calcutta, 51/2 Hazra Road Kolkata-700019** |
| 4. UGC APPROVAL LETTER NO. AND DATE | : **F. 42-721/2013(SR) dated 21.03.2013** |
| 5. DATE OF IMPLEMENTATION | : **01.04.2013** |
| 6. TENURE OF THE PROJECT | : **01.04.2013 to 31.03.2016** |
| 7. TOTAL GRANT ALLOCATED | : **Rs. 12, 55, 980.00** Twelve lakh fifty five thousand Nine hundred Eighty only**)** |
| 8. TOTAL GRANT RECEIVED | : **Rs. 11, 40,262.00 (**Eleven lakh Forty thousand Two hundred Sixty Two only**)** |
| 9. FINAL EXPENDITURE | :Rs. **12, 94, 721.00 (** Twelve lakh Ninety Four thousand Seven hundred Twenty One only**)** |
| 10. TITLE OF THE PROJECT | : **Genetic diversity among genotypes and molecular linkage map construction in sesame (Sesamum *indicum* L.)** |

**11. Objectives of the project**

The bridge between the genetic map and assembled genome sequence then enables the suggestion of candidate genes corresponding to QTL. Finally, it forms a vital tool in marker-assisted plant breeding programs, enabling plant breeders to develop in a targeted fashion new plant varieties in response to demands such as increased yield and resistance to pests and pathogens. Genetic linkage maps can provide a more direct method for selecting desirable genes via their linkage to easily detectable molecular markers. As breeding in sesame is a long process and the speed and precision of breeding can be improved by the development of genetic linkage maps, hence, construction of linkage map is momentous in sesame as genetic linkage maps can facilitate the development of diagnostic markers for polygenic traits and the identification of genes controlling complex phenotypes. Further, limited work has been done in sesame either to determine genetic diversity or construction of linkage map.

i. Development and identification of EST-SSR, SSR and SCAR markers polymorphic nature.
ii. Study of Morphology of different genotypes
iii. Study on genetic diversity of sesame genotypes exotic as well as Indian origin.
iv. Screening of parents for polymorphism in markers and genotyping the mapping population.
v. Development of mapping population
vi. Linkage analysis.

12. Whether objectives were achieved : Yes, the details of the results are provided in separate "Final Report".

## 13. Achievements from the project:

Oilseeds constitute a very important group of commercial crops in India. The oil extracted from oilseeds form an important item of our diet and are used as raw materials for manufacturing large number of items like paints, varnishes, hydrogenated oil, soaps, perfumery, lubricants, etc. Sesame is considered as a nutritious oilseed crop being rich source of protein (18–25 %), carbohydrate (13.5 %), minerals and healthy polyunsaturated fatty acid. Sesame oil is favored as a media of cooking by Indians and Africans. Presence of sesamol, a unique anti-oxidant and more poly-unsaturated fatty acid, have made it to 'queen of oilseed crop'. In spite of being the first oilseed crop known to man and momentous history, sesame is typically a neglected crop. Sesame needs improvement in productivity of crops with high oil content and application marker assisted breeding. As information about SSR marker is not much available in this crop the project deals

with identification of EST SSR (genic) markers, application of markers in association mapping and also development of linkage map by genotyping in mapping population.

To carry out linkage map we have developed the mapping population from the cross combination (Savitri with EC-335004) with distinctly different ideotypes with respect to seed size, seed colour and branching habit. From the mapping population we have identified 3 lines which have higher concentration of Iron, Zinc and protein with high yield. These 3 lines can be developed into commercial varieties in advance generation after multi location trial.. 35 EST-SSR markers have been identified to be polymorphic which can be used in Marker assisted selection. These developed markers were used in association mapping (AM), also known as linkage disequilibrium (LD) study. In this study we have mapped the individual protein content and yield attributes in sesame with help of 35 Polymorphic EST-SSR markers and 50 diverse genotypes. The genotypes were distinguished into 5 population belonging to different location. Interestingly, landraces formed a distinct group in population diversity analysis. Association mapping (AM) was applied to sesame germplasm collection in order to provide new insight into the genetic control of total and individual protein content and yield attribute. In the present study, the observed LD value ($r^2$) rapidly decreased when the genetic distance was less than 10 cM. The speed of population LD decay was 8.58 or 5.76 cM for r2 > 0.1 or 0.2, respectively. We selected markers that were spaced approximately 10 cM apart from the frame linkage map Because of the shortage of polymorphism markers, there were some gaps of more than 15 to 46.8 cM along the 26 chromosomes.

## 14. Summary of the findings

In this study we have developed the mapping population from the cross (Savitri and EC-335004). 239 EST-SSR markers have been developed obtaining deposited sequence data from National Centre for Biotechnology Information (NCBI) web site and following bio-informatics tools like vecScreen, CAP3 and MISA. Finally through primer3 tools, the primers have been designed. These developed EST-SSR markers have been tested for polymorphism on diverse genotypes of sesame. 35 EST-SSR markers have been identified to be polymorphic and good for the association mapping (AM),also known as linkage disequilibrium (LD) study. In this study we have mapped the individual protein content and yield attribute in sesame with help of 35 Polymorphic EST-SSR markers and 50 diverse genotypes. Association mapping (AM) was applied to sesame germplasm

collection in order to provide new insight into the genetic control of total and individual protein content and yield attribute.

In the present study, Fourteen markers tolerated the FDR test in one or more environments, including 12 for protein content and yield attribute. Two marker loci presented moderate-to-strong or strong-to-very strong evidence for association indifferent environments. Six markers for oil traits passed the permutation test at the 0.05 level in the GLM analysis.

In the present study, 9.36% of the marker pairs showed significant LD values, while 18.90% of collinear and 8.90% of non-collinear marker pairs showed significant LD. Among the collinear marker pairs, 29.2% showed LD values ($r^2$) greater than 0.2.For the non-collinear marker pairs, this ratio was 0.5%. Further examination of the LD data revealed that approximately 80.5% of moderate LD (0.2 < r2 < 0.4) and 91.5% of strong LD (r2 > 0.4) was caused by linkage. Our results also showed that approximately 43.6%of moderate LD ($r^2$> 0.1) was caused by other factors in this panel.

In the present study, the observed LD value ($r^2$) rapidly decreased when the genetic distance was less than 10 cM. The speed of population LD decay was 8.58 or 5.76 cM for r2 > 0.1 or 0.2, respectively. We selected markers that were spaced approximately 10 cM apart from the frame linkage map Because of the shortage of polymorphism markers, there were some gaps of more than 15 to 46.8 cM along the 26 chromosomes. Although more markers are needed to conduct genome-wide association analyses (GWAS) of complex traits, the size of the LD blocks would guarantee that the identified SSR markers would be sufficient for MAS in sesame breeding programs because increasing the number of markers per chromosome does not necessarily result in a stronger response to selection, particularly at a shorter distance between markers.

## 15. Contribution to the society

Sesame is one of the most ancient and important oilseed crops grown and used by mankind. Sesame is considered as a nutritious oilseed crop being rich source of protein (18–25 %), carbohydrate (13.5 %), minerals and healthy polyunsaturated fatty acid. Sesame has a relatively superior oil quantity as well as quality in comparison to many major oil crops. The oil content ranges from 34.4 to 59.8% but is mostly about 50% of seed weight. Nutritional variation in black and white sesame wherein protein, fat, zinc, copper, sodium, magnesium, glucose, sucrose,

maltose, Vitamin C, E and K being higher in white seeds. Presence of sesamol, a unique anti-oxidant and more poly-unsaturated fatty acid, have made it to 'queen of oilseed crop'. Horizontal expansion of any crop depends congenial climate of production, soil factor and renumerative price of the crop, so that the farmers can grow it more. Sesame productivity in India is low compared to other sesame producing countries. Genetic upgradation of any crop primarily depends on utilization of existing genetic resource In spite of being the first oilseed crop known to man and momentous history, sesame is typically a neglected crop. As a consequence of this, the use of molecular techniques for the improvement of sesame is very limited.

In this study we have developed the mapping population from the cross (Savitri and EC-335004). From this mapping population we have identified 3 lines which have higher concentration of Iron, Zinc and protein with high yield. These 3 lines can be developed into commercial varieties after the stability of segregation. 35 EST-SSR markers have been identified to be polymorphic which can be used in Marker assisted selection. This Marker assisted selection will eradicate the time consumption for the selection process. It will be more accurate and efficient selection of specific genotypes and may lead to accelerated variety development.

16. Whether any Ph.D. enrolled/produced out of the project : Yes , One Ph.D Student is enrolled at University of Calcutta

17. NO. OF PUBLICATIONS OUT OF THE PROJECT : 4 (Reprints attached)

**Research paper published:**


1. **A. Iqbal** and T Dasgupta, Genetic Estimates of Morphological Traits and Phenotypic Diversity in Core Collections of Sesame (Sesamum indicum L.) *Indian Agriculurist*, Vol.5, 2015, 61-69.
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Dgupta 22/05/18
(PRINCIPAL INVESTIGATOR )

Dr. Tapash Dasgupta
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Registrar
University of Calcutta

Annexure – X

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

# EVALUATION REPORT

It is certified that the final report of the Major Research Project entitled " Genetic diversity among genotypes and molecular linkage map construction in sesame (Sesamum *indicum* L.)" by Prof Tapash Dasgupta, Dept. of Genetics and Plant Breeding, Institute of Agricultural Science, University of Calcutta has been assessed by the 'Evaluation Committee' consisting the following members for final submission to the University Grants Commission, New Delhi.

**Details of Expert Committee**

1. Prof. Pranab Talukdar, Dept of Plant Breeding, Assam Agricultural University, Jorhat, Assam
2. Prof. Subhra Mukerjee, Dept of Genetics and Plant Breeding, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal

**The Final Report is found to be satisfactory.**

Prof. Pranab Talukdar,
Professor
Deptt. of Plant Breeding &Genetics
A. A U., Jorhat-13

Prof. Subhra Mukerjee,
Dr. Subhra Mukherjee
Professor
Deptt. of Genetics & Plant Breeding, F/Ag.
Bidhan Chandra Krishi Viswavidyalaya
Mohanpur-741252, Nadia, W.B.

(REGISTRAR)
(Seal)

Registrar
University of Calcutta

To

The Hon'ble Vice Chancellor
University of Calcutta,
Kolkata – 700019.

OFFICE OF THE VICE CHANCELLOR
17 NOV 2017
Letter No. 65/1117
UNIVERSITY OF CALCUTTA

Dated: 14.11.2017

7379/1117
PVC (B.A & F).....
Dated: 22.11.17

**Sub: Request for the approval of evaluation committee for UGC MRP (final report submission)**

Respected Madam,

I would like to inform you that I have completed a **UGC Major Research Project** titled "**Genetic diversity among genotypes and molecular linkage map construction in sesame (Sesamum *indicum* L.)" [Ref No. : F. 42-721/2013(SR) dated 21.03.2013].** Before submission of the final report to the funding agency, the report is required to be evaluated by two experts not belonging to the implementing institution.

In this regard, I am proposing the name of two expert members for evaluation of the final report. I would be obliged if you approve the same and do the needful in this regard.

With warm regards,
Yours sincerely

Dgupta 14.11.2017

Prof. Tapash Dasgupta
Professor (Retired)
Dept. of Genetics and Plant Breeding,
Institute of Agricultural Science
University of Calcutta

PVC (F)
SCB
22.11.17

Name of Expert:

1. **Prof. Pranab Talukdar,** Dept of Plant Breeding, Assam Agricultural University, Jorhat, Assam
2. **Prof. Subhra Mukerjee,** Dept of Genetics and Plant Breeding, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal

Forwarded
16.11.17

HEAD
Dept. of Genetics & Plant Breeding
Institute of Agricultural Science
University of Calcutta
51/2 Hazra Road, Kol : 700 019

Approved
30/11/17

## Summary of the project report ( F. 42-721/2013(SR) dated 21.03.2013)

### GENETIC DIVERSITY AMONG GENOTYPES AND MOLECULAR LINKAGE MAP CONSTRUCTION IN SESAME ( *Sesamum indicum* L.)

In this study we have developed the mapping population from the cross (Savitri and EC-335004). 239 EST-SSR markers have been developed obtaining deposited sequence data from National Centre for Biotechnology Information (NCBI) web site and following bio-informatics tools like vecScreen, CAP3 and MISA. Finally through primer3 tools, the primers have been designed. These developed EST-SSR markers have been tested for polymorphism on diverse genotypes of sesame. 35 EST-SSR markers have been identified to be polymorphic and good for the association mapping (AM),also known as linkage disequilibrium (LD) study. In this study we have mapped the individual protein content and yield attribute in sesame with help of 35 Polymorphic EST-SSR markers and 50 diverse genotypes. Association mapping (AM) was applied to sesame germplasm collection in order to provide new insight into the genetic control of total and individual protein content and yield attribute.

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than 15 to 46.8 cM along the 26 chromosomes. Although more markers are needed to conduct genome-wide association analyses (GWAS) of complex traits, the size of the LD blocks would guarantee that the identified SSR markers would be sufficient for MAS in sesame breeding programs because increasing the number of markers per chromosome does not necessarily result in a stronger response to selection, particularly at a shorter distance between markers.

# OBJECTIVES

The bridge between the genetic map and assembled genome sequence then enables the suggestion of candidate genes corresponding to QTL. Finally, it forms a vital tool in marker-assisted plant breeding programs, enabling plant breeders to develop in a targeted fashion new plant varieties in response to demands such as increased yield and resistance to pests and pathogens. Genetic linkage maps can provide a more direct method for selecting desirable genes via their linkage to easily detectable molecular markers. As breeding in sesame is a long process and the speed and precision of breeding can be improved by the development of genetic linkage maps, hence, construction of linkage map is momentous in sesame as genetic linkage maps can facilitate the development of diagnostic markers for polygenic traits and the identification of genes controlling complex phenotypes. Further, limited work has been done in sesame either to determine genetic diversity or construction of linkage map.

i. Development and identification of EST-SSR, SSR and SCAR markers polymorphic nature.
ii. Study of Morphology of different genotypes
iii. Study on genetic diversity of sesame genotypes exotic as well as Indian origin.
iv. Screening of parents for polymorphism in markers and genotyping the mapping population.
v. Development of mapping population
vi. Linkage analysis.

## Achievements from the project :

Oilseeds constitute a very important group of commercial crops in India. The oil extracted from oilseeds form an important item of our diet and are used as raw materials for manufacturing large number of items like paints, varnishes, hydrogenated oil, soaps, perfumery, lubricants, etc. Sesame is considered as a nutritious oilseed crop being rich source of protein (18–25 %), carbohydrate (13.5 %), minerals and healthy polyunsaturated fatty acid. Sesame oil is favored as a media of cooking by Indians and Africans. Presence of sesamol, a unique anti-oxidant and more poly-unsaturated fatty acid, have made it to ‘queen of oilseed crop’. In spite of being the first oilseed crop known to man and momentous history, sesame is typically a neglected crop.

Sesame needs improvement in productivity of crops with high oil content and application marker assisted breeding. As information about SSR marker is not much available in this crop the project deals with identification of EST SSR (genic) markers, application of markers in association mapping and also development of linkage map by genotyping in mapping population.

To carry out linkage map we have developed the mapping population from the cross combination (Savitri with EC-335004) with distinctly different ideotypes with respect to seed size, seed colour and branching habit. From the mapping population we have identified 3 lines which have higher concentration of Iron, Zinc and protein with high yield. These 3 lines can be developed into commercial varieties in advance generation after multi location trial.. 35 EST-SSR markers have been identified to be polymorphic which can be used in Marker assisted selection. These developed markers were used in association mapping (AM), also known as linkage disequilibrium (LD) study. In this study we have mapped the individual protein content and yield attributes in sesame with help of 35 Polymorphic EST-SSR markers and 50 diverse genotypes. The genotypes were distinguished into 5 population belonging to different location. Interestingly, landraces formed a distinct group in population diversity analysis. Association mapping (AM) was applied to sesame germplasm collection in order to provide new insight into the genetic control of total and individual protein content and yield attribute. In the present study, the observed LD value ($r^2$) rapidly decreased when the genetic distance was less than 10 cM. The speed of population LD decay was 8.58 or 5.76 cM for r2 > 0.1 or 0.2, respectively. We selected markers that were spaced approximately 10 cM apart from the frame linkage map Because of the shortage of polymorphism markers, there were some gaps of more than 15 to 46.8 cM along the 26 chromosomes.

## Major findings:

i. Assessment of genetic diversity of local as well as exotic sesame genotypes have been done
ii. In sesame there is dearth of availability of simple sequence repeat markers which are this point of time a most abundant, significance reproducible, easily scoring and cost effective.

iii. The present study helped to develop a number of EST-SSR primers marker which will ultimately help in construction of linkage map and mark assisted selection.

iv. Detailed genetic analysis of qualitative and quantitative traits through construction of map.

v. From the mapping population we have identified 3 lines which have higher concentration of Iron, Zinc and protein with high yield. These 3 lines can be developed into commercial varieties in advance generation after multi location trial.

vi. 35 EST-SSR markers have been identified to be polymorphic which have be used in Marker assisted selection.

vii. In this study we have mapped the individual protein content and yield attributes in sesame with help of 35 Polymorphic EST-SSR markers and 50 diverse genotypes.

viii. Association mapping (AM) was applied to sesame germplasm collection in order to provide new insight into the genetic control of total and individual protein content and yield attribute.

## Contribution to the society

Sesame is one of the most ancient and important oilseed crops grown and used by mankind. Sesame is considered as a nutritious oilseed crop being rich source of protein (18–25 %), carbohydrate (13.5 %), minerals and healthy polyunsaturated fatty acid. Sesame has a relatively superior oil quantity as well as quality in comparison to many major oil crops. The oil content ranges from 34.4 to 59.8% but is mostly about 50% of seed weight. Nutritional variation in black and white sesame wherein protein, fat, zinc, copper, sodium, magnesium, glucose, sucrose, maltose, Vitamin C, E and K being higher in white seeds. Presence of sesamol, a unique anti-oxidant and more poly-unsaturated fatty acid, have made it to 'queen of oilseed crop'. Horizontal expansion of any crop depends congenial climate of production, soil factor and renumerative price of the crop, so that the farmers can grow it more. Sesame productivity in India is low compared to other sesame producing countries. Genetic upgradation of any crop primarily depends on utilization of existing genetic resource In spite of being the first oilseed crop known to man and momentous history, sesame is typically a neglected crop. As a consequence of this, the use of molecular techniques for the improvement of sesame is very limited.

In this study we have developed the mapping population from the cross (Savitri and EC-335004). From this mapping population we have identified 3 lines which have higher concentration of

Iron, Zinc and protein with high yield. These 3 lines can be developed into commercial varieties after the stability of segregation. 35 EST-SSR markers have been identified to be polymorphic which can be used in Marker assisted selection. This Marker assisted selection will eradicate the time consumption for the selection process. It will be more accurate and efficient selection of specific genotypes and may lead to accelerated variety development.

**Appendix 1**
**Final Report of the Work**

Project Title:
**GENETIC DIVERSITY AMONG GENOTYPES AND MOLECULAR LINKAGE MAP CONSTRUCTION IN SESAME (Sesamum *indicum* L.)**

# 1.1 Introduction to sesame:

Sesame (*Sesamum indicum*) is a flowering plant in the genus *Sesamum*, also called *benne* or *Til* is one of the most ancient oilseed crops known and used by mankind. It is an important oilseed crop in the tropics and warm sub tropics. It is extensively naturalized in tropical regions around the world and is cultivated for its edible seeds which grow in capsules. It was cultivated and domesticated on the Indian subcontinent during Harappan and Anatolian eras over 4,000 yrs ago. (Bedigian and Van der Mesen, 2003).Sesame has one of the highest oil contents of any seed. With a rich, nutty flavor, it is a common ingredient in cuisines across the world. Like other nuts and foods, it can trigger allergic reactions in some people. Sesame is so ancient that it is almost impossible to say with any degree of accuracy where and when this domestication took place and there is some disagreement on this point till today. In India sesame is grown in three seasons; *kharif* (rainy), semi *rabi* or post *kharif* and summer or pre *kharif*. Sesame crop has a wide attributes', such as: short growth period, photo-insensitivity, enabling the crop to rise in any season; higher oil percentage, drought evading ability, easy crushing and extracting of oil, higher quality of edible oil because of its higher degree of poly-unsaturated fatty acid (PUFA) content. On the other side this crop does well under a wide range of soil and climatic condition particularly under dry farming condition. Sesame is a highly drought tolerant crop and grows well in most kind of soils, regions and is also well suited to different crop rotations. In reality, sesame is mostly grown under moisture stress with low management input by small holders.

**Figure 1: Different seed code colour in sesame**

## 1.2 Agroecology:

Sesame is a crop of the tropics and subtropics. With newer cultivars and summer plantings its range has extended into the temperate areas. Sesame is cultivated mainly between 25°S and 25°N, but extends further to 40°N in China, Russia and the United States, 30°S in Australia and

35°S in South America. It is grown from sea level to 1,800 m altitude. The crop has an optimal day temperature of 25–27°C, below 20°C growth is retarded and below 10°C germination is suppressed Sesame requires 90–120 frost free days. Sesame is adaptable to many soil types, but it thrives best on well-drained, fertile soils of medium texture and with pH ranging from 5.5 to 8.0, but most cultivars are intolerant of salinity (Lim *et al.*, 2012).

## 1.3 Oil content and fatty acid composition of sesame seeds

Sesame has a relatively superior oil quantity as well as quality in comparison to many major oil crops. The oil content ranges from 34.4 to 59.8% but is mostly about 50% of seed weight (Ashri, 1998; Azeez and Morakinyo, 2011) reported that seed oil content was 53.23–55.12% in cultivated while 53.35–58.56% in wild accessions. Values of up to 63.2% have been reported in some varieties by Baydar *et al*., (1999). Both genetic and environmental factors influence the oil content in sesame. Were *et al*.,(2006) could establish correlation between fatty acid and oil concentration in a three year study of sesame. They reported that oil content correlated negatively with palmitic and linoleic acids, and positively with stearic and oleic acids. Late maturing cultivars are reported to have higher oil content than early ones (Yermanos *et al.*, 1972; Uzun *et al*., 2007) observed that indeterminate cultivars accumulated more oil than determinate ones. Variation also occurs between capsules at different positions on the same plant, such that seeds from the basal capsules on the main stem contain more oil than those located towards the apex and on side branches (Mosjidis and Yermanos, 1985;). Black seeded cultivars often have lower oil content than brown or white seeded ones, indicating a possible linkage between oil content and the pathway that contributes to the seed coat colour. Kamal-Eldin and Appelqvist (1994) have attributed the low oil content in black seeded sesame to a high amount of crude fibre in the seed coat. Black seed coat is usually thicker than lighter coloured ones. Philip(2011) reported nutritional variation in black and white sesame wherein protein, fat, zinc, copper, sodium, magnesium, glucose, sucrose, maltose, Vitamin C, E and K being higher in white seeds.
The genus sesame has limited variability in the seed fatty acid proportions (Kamal-Eldin et al., 1992). The seed fatty acid composition varies considerably among the different cultivars of sesame worldwide (Yermanos *et al*., 1972; Brar, 1979; Baydar *et al*., 1999). The oil contains four major fatty acids namely, palmitic, stearic, oleic and linoleic acids, along with small quantities of vaccenic, linolenic, arachidic, behenic and eicosenoic acids (Weiss, 1983; Kamal Eldin *et al*., 1992;). Oleic and linoleic acids occur in nearly equal amounts, constituting about 85% of the total fatty acids. Sesame treated with mutagens shows fatty acid variation having saturated fatty acids higher than control besides lower concentration of polyunsaturated fatty acids. As regards the oleic acid, the high yielding/branched mutant was revealed the highest oleic acid content (Savant and Kothekar, 2011).

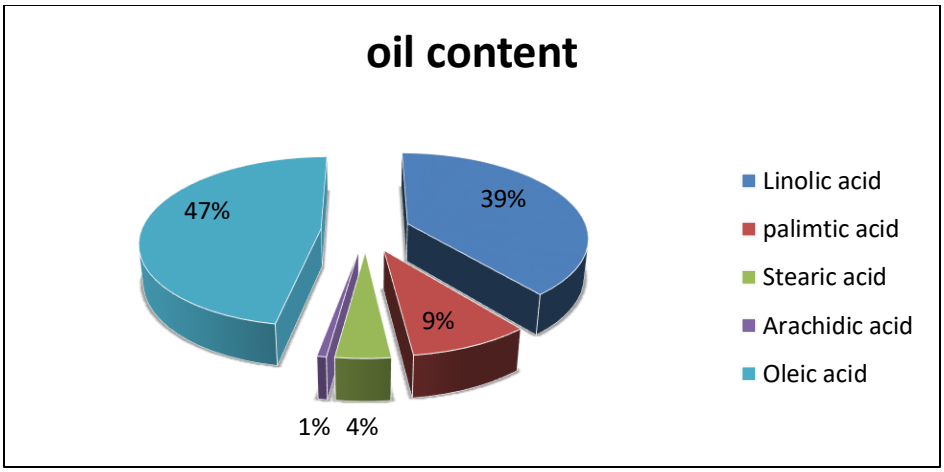



**Figure: 1.3.1 oil content in sesame**

Genotypes with exceptionally high (> 60%) oleic or linoleic acid are rare (Baydar *et al.*, 1999. and Uzun *et al.*2007) found differences in stearic, oleic and linoleic acids between determinate and indeterminate cultivars. The Cultivars which growth are determinate in nature generally have higher stearic and oleic acids, and lower linoleic acid compared to indeterminate ones. Capsule position on the plant also affects the relative quantities of the fatty acids; palmitic, stearic and oleic acids tend to increase up the stem while linoleic acid decreases (Brar, 1977). The fatty acid composition is strongly influenced by environmental factors. Linoleic acid content has been reported to increase under cool growing conditions (Uzun *et al.*, 2007).

Recent studies have shown that sesame oil is beneficial in lowering cholesterol levels and hypertension (Sankar *et al.*, 2004; Frank *et al.*, 2005), and reducing the incidence of certain cancers (Hibasami *et al.*, 2000). These health enhancing effects of sesame oil are explained by the low level of saturated fatty acids and high levels of PUFA. Moreover, the Sesamin is known to enhance the availability and functioning of vitamin E (tocopherol). An elevated concentration of tocopherol in the blood is associated with reduced risk of heart disease and some cancers e.g. of the upper gut. Thus, sesame oil could be beneficial for enhancing health by improving the vitamin E levels in the body (Frank et al., 2005).

### 1.3.1 Sterols:

Sesame oil is relatively high in unsaponifiable matter (2%) compared with other vegetable oils. The unsaponifiable matter includes sterols, triterpenes and triterpene alcohols, tocopherols, and sesame lignans. Sterols are present in vegetable oils in free form or as sterol esters, sterol glucosides, or esterified steryl glucosides, but free sterols and sterol esters are often the dominant forms. Among the three classes of sterols, desmethylated sterol is the major one (85-89% of total sterols) followed by monomethylated (9-11%) and dimethylated (2-4%) sterols in sesame oil.

### 1.3.2 Tocopherols:

Oxidative stability of Sesame oil is well known; one of the reasons for this extra-stability is attributed to its tocopherol content. The total tocopherol content of sesame oil ranges from 330-mg/kg to 1010-mg/kg oil.

### 1.3.3 Protein:

The protein content of sesame seed is approximately 25% with a range of 17-31% depending on the source of the seed. Sesame protein is low in lysine (3.1% protein), but it is rich in sulphur containing amino acids methionine and cystine (6.1%), which are often the limiting amino acids in legumes. Because of its characteristic amino acid composition, sesame seed protein is regarded as an excellent protein source for supplementing many vegetable proteins such as soybean and peanut to increase their nutritional value. The dark seeds had significantly higher oil and lower protein contents than the white coloured sesame seeds. Oil content was found to be 47.02% and 49.07% in the white and dark coloured seeds, respectively.

### 1.3.4 Sesame lignans and lignin glycosides:

Lignans are compounds formed by oxidative coupling of r-hydroxyphenylpropane. They are widely distributed in all parts of plants. Oilseeds such as sesame and flaxseed are well known to contain abundant lignans (Wanasundara et al. 1997).Two types of lignan compounds existed in sesame seeds, the oil soluble lignans and the water soluble lignan glycosides. In raw sesame seed, sesamin and sesamolin are the two major lignans. Sesamin has been found in other plants, whereas sesamolin is the distinct characteristic of sesame and has not been found in plants other than *Sesamum*.

## 1.4 Nutrient data for sesame seed

Cooney et al. (2001) revealed that intake of food made from sesame seed, containing gamma tocopherol, significantly elevated its level in the serum. Gamma-tocopherol promotes vitamin E Activity that is believed to prevent cancer and heart disease (Cooney et al. 2001).

Sesame oil as a pharmaceutics aid (Jellin et al., 2000) is often used for medication of gum treat toothaches, relive anxiety or insomnia, or as an antibacterial mouthwash bydisease, Chinese and Indian in the past (Annussek, 2001; Morris, 2002).

**Table: 1.4.1- Nutritional values in sesame seed**

| Nutrient | Unit | Value/100g | Nutrient | Unit | Value/100g |
|---|---|---|---|---|---|
| **Water** | G | 3.75 | Vitamin C, total ascorbic acid | Mg | 0 |
| **Energy** | Kcal | 613 | Thiamin | Mg | 0.699 |
| **Protein** | G | 20.45 | Riboflavin | Mg | 0.09 |
| **Total lipid (fat)** | G | 61.21 | Niacin | Mg | 5.8 |
| **Carbohydrate, by difference** | G | 11.73 | Vitamin B-6 | Mg | 0.4 |
| **Fiber, total dietary** | G | 11.6 | Folate, DFE | µg | 115 |
| **Sugar, total** | G | 0.48 | Vitamin B-12 | µg | 0 |
| **Zinc, Zn** | Mg | 6.73 | Vitamin A, RAE | µg | 3 |
| **Calcium, Ca** | Mg | 60 | Vitamin A, IU | lU | 66 |

| | | | | | |
|---|---|---|---|---|---|
| **Iron, Fe** | Mg | 6.36 | Vitamin E(alpha-tocopherol) | Mg | 1.68 |
| **Magnesium, Mg** | Mg | 345 | Vitamin D(D2+ D3) | µg | 0 |
| **Phosphorus, P** | Mg | 667 | Vitamin D | lU | 0 |
| **Potassium, K** | Mg | 370 | Vitamin K (phylloquinone) | µg | 0 |
| **Sodium, Na** | Mg | 47 | Fatty acids, total saturated | G | 9.055 |
| **Caffeine** | Mg | 0 | Fatty acids, total monounsaturated | G | 23.924 |
| **Cholesterol** | Mg | 0 | Fatty acids, total polyunsaturated | G | 25.491 |

## 1.5 Production of Sesame:

### 1.5.1 Production of Sesame in world:

Sesame ranks eighth in the world production of edible oil seeds and is grown over 70 countries around the world. Among the five continents, Asia has the highest area of harvest (4.6 million ha), which produces 2 million MT of sesame seed annually. Europe has the lowest quantity of seed production (only 0.057% of the world total) but the highest yield (4968.5 kg/ha) of sesame seed. This yield is ten times that of Asia where more than 63.2% of world's sesame seeds are produced. Africa, the origin of sesame seed, is the second largest sesame-producing continent 32.4% of the total production. It has, however, the lowest yield (only 328 kg/ha) of sesame seed.

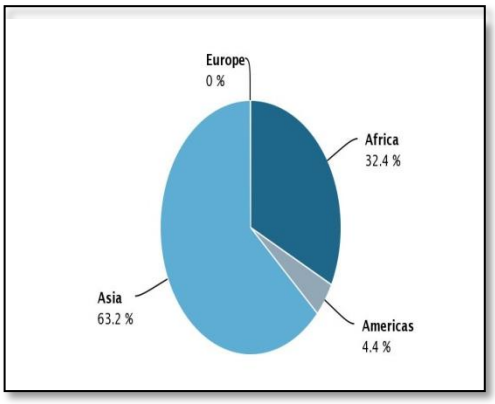



**Figure 1.5.1.1 Production of sesame in the different continent**

At present India and China are the world's largest producers of sesame, followed by Myanmar, Sudan, Nigeria, Uganda, Pakistan, Tanzania, Ethiopia, Guatemala and Turkey. World production does fluctuate due to local economic, crop production disturbance and weather conditions.

India is the largest producer of sesame in the world. It also ranks first in the world in terms of in sesame-growing area (24%) and also in terms of export (40%) (FAO Statistics Division, 2010; Raikwar and Srivastva, 2013). But the production of sesame in India is not constant. India has

produced around 893000 tons in 2010 but after that the production of sesame has been declined in recent years, till 2013 the production of sesame has been in declining phase but in 2014it increased but in 2015 the production again decreased to 79000 tonnes but again in 2016 it decreased.

Area of harvest of sesame is also not constant 9in India in last 10 years. It also shows the fluctuation. In recent times area is decreased to 1630000 ha in comparison to 2079280ha in 2010.

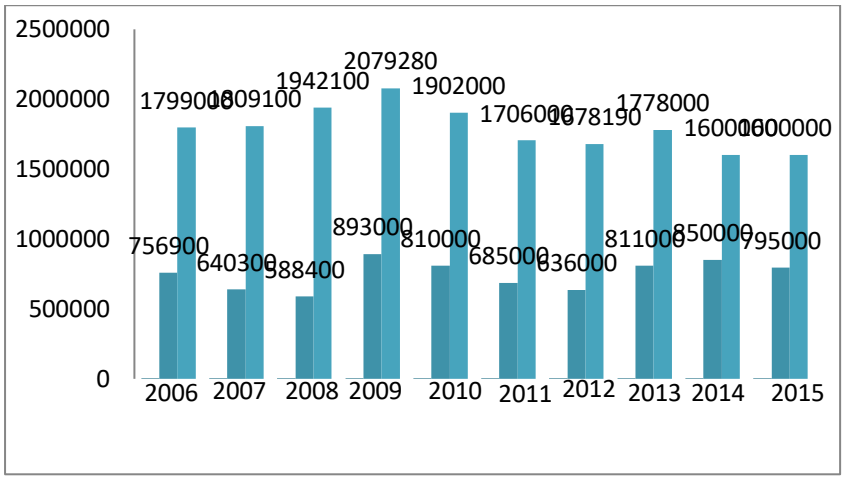



**Figure: 1.5.1.2. Summary of sesame Production and Area.**

With a population now approaching 1.3 billion, Indian ranks second in total population closely preceded by China, India is expected to reach the top position, population wise in near future, presently, nearly 58 percent of population depends principally on agriculture as a profession. Agriculture sector in India area in India (159.7 million hectare) is second largest in the world, preceded by United state, albeit gross irrigated crop area of India (82.6 million hectare) occupies the top position in the world.

Among the agricultural crops, cereals and oilseeds are two major components while comparing production figure. After cereals, oilseeds are the second largest agricultural commodity in India. The Technology Mission on oilseeds, launched by Government of India in 1986, escalated the oilseeds production from 10.83, million tonnes in1985-86 to 24.35 million in 1996-97 through horizontal and vertical expansion of the crop. The productivity boosted up to 926 Kg/Ha in 1996-97 from 684 Kg/Ha in 1985-86.

## 1.7. **Demand and production of sesame seed**

This quantum jump in oilseed production popularity referred as “Yellow revolution” in the late eighties and early nineties, turned up total scenario of the status of India to an export promoting country during 1989-90 from deficit productivity import dependent country in 1989-90. India earned foreign exchange of Rs. 2800 crores through sesame and it is worth mentioning in terms of financial contribution. India export sesame to a number of countries with significant quantity.

The total production of sesame in India generates a great impact on world market. At present the annual growth rate being around 6% the demand of oilseed after next ten years may reach almost double of the current supply.

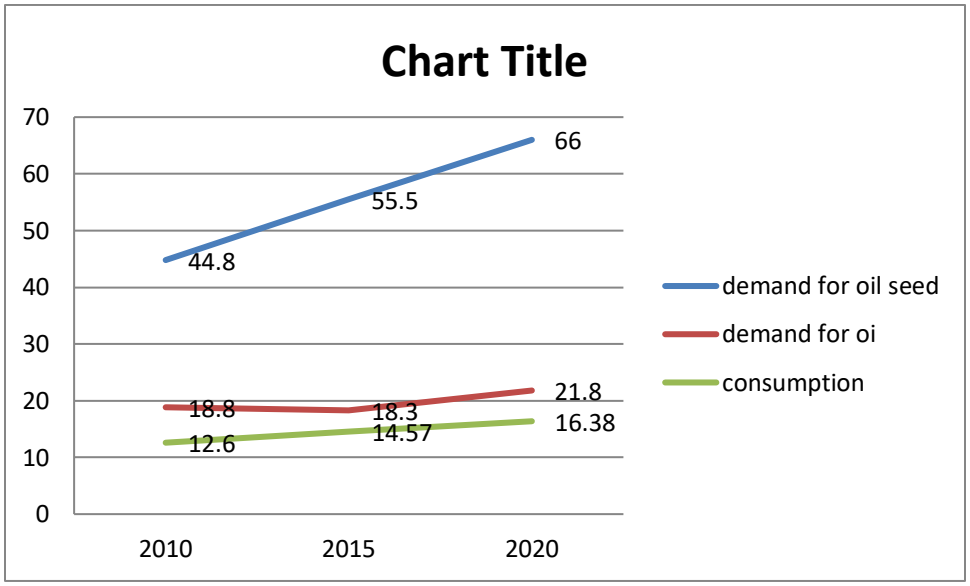



**Figure 1.7.1 Demand and production of sesame seed**

## 1.8: Problems of Sesame:

In Indian, the potential of sesame is considered to be high but production is below expectation. The low production is due to a number of reasons such as low inputs and poor management (e.g low or non-fertilization, irrigation, pest control etc), occurrence of biotic and abiotic stresses and more importantly, lack of an appropriate breeding program. Major problems of Sesame can be summarized as follows:

- Poor yield due to lack of improved cultivars
- Low harvest index and indeterminate growth habit
- Asynchronous capsule ripening that accentuates seed shattering
- Dehiscent capsules leading to yield loss
- Cultivation in marginal and sub-marginal lands using inappropriate production technologies (broadcast method of sowing, little or no use of fertilizers & untimely weed management) (Khalaque & Begum, 1991).
- Severe biotic stresses, such as: Bacterial blight, Phyllody, Fusarium wilt, Powdery mildew, *Alternaria* leaf spot and *Cercospora* leaf spot.
- So far very less attention has been paid for the development of high yielding appropriate plant type capable of maintaining high performance in yield, oil and protein profile.

The above mentioned constraints to the productivity of sesame pose the need for concerted efforts for sesame crop improvement.

## 2.1 Materials:

Seventy (50) genotypes of sesame (Table- 2.1.1), collected from different oil seed crops research centers like Indian Institute of Oil Research, Oilseeds Research station junagadh, Oilseeds Research Station (TNAU), Jawaharlal nehru krishi vishwa vidyalaya (JNKVV) and Pulses & Oilseeds Research Station, Beraham Pur, Murshidabad, West Bengal. **State check variety *SAVITRI* and national check variety GT-10** was also included in the experiment.

**Table-2.1.1: List of experimental material**

| SERIAL NO. | NAME | Local land race/ variety | CENTRE OF ORIGIN | SALIENT FEATURES/ OTHER INFORMATION |
|---|---|---|---|---|
| 1 | RT348 | HYV | Local Rajasthan | White seed; high 1000 seed weight |
| 2 | TMV 4 | HYV | TNAU, Coimbatore, Tamil Nadu | Light brown seed, Moderately resistant to Macrophomina |
| 3 | UMA | HYV | OUAT, Bhubaneswar, (Oddisha) | Beige seed, Tolerant to stem/ root rot and phyllody |
| 4 | CUMS 04 | HYV | West Bengal, (Mutant line) | Brown seed |
| 5 | RAMA | HYV | Pulse and oil seed research station, Berhampore, WB | Dark brown seed, Resistant to Alternaria, Phyllody and hairy caterpillar |
| 6 | GT-10 | HYV | ARS, GAU, Amreli(Gujrat) | Black seed. High oil content |
| 7 | V-12 | Land race | West Bengal, local malda | Dark green narrow leaves, tri-lobed at maturity; 4/6/8 locular capsules, deep brown seed |
| 8 | OSC-593 | HYV | Bhubaneswar, (Oddisha) | White seed; short plant height; less branching |
| 9 | V-10 | Land race | West Bengal, malda local | Deep brown seed |
| 10 | AMRIT | HYV | OUAT, Bhubaneswar, (Oddisha) | Light brown seed, Moderately resistance to powdery mildew |
| 11 | B9 | HYV | Local West Bengal, 24 paragana south | Brown seed |
| 12 | RT-54 | HYV | ARS, SKRAU, Mandore, (Rajasthan) | Light brown seed, Resistance to leaf blight, Tolerant to alternaria |
| 13 | SAVITRI | HYV | Pulse and oil seed | Light brown seed, Tolerant to |

| SERIAL NO. | NAME | Local land race/ variety | CENTRE OF ORIGIN | SALIENT FEATURES/ OTHER INFORMATION |
|---|---|---|---|---|
| | | | research station, Berhampore, (WB) | Macrophomina |
| 14 | CUMS 9-1 | HYV | Mutant line, West Bengal | Mixture of brown and olive green seed |
| 15 | TILLOTTAMA | HYV | Pulse and oil seed research station, Berhampore, WB | Dark brown seed, Tolerant to Macrophomina, Phyllody and Bihar hairy caterpillar. |
| 16 | NIC 8316 | HYV | Indegenous collection | Beige seeds |
| 17 | NIRMALA | HYV | OUAT, Bhubaneswar, (Oddisha) | Light brown seed, Tolerant to phyllody, Wilt, Resistant to bacterial leaf spot, Powdery moldew, Moderately resistant to stem/ root rot and Alternaria |
| 18 | TKG 355 | HYV | Local land race | White seed |
| 19 | IC – 131490 | Land race | Indigenous variety | Medium brown seed |
| 20 | IC – 14331 | Land race | Indigenous variety | White seed |
| 21 | IC – 14053 | Land race | Indigenous variety | Medium brown seed |
| 22 | IC – 43033 | Land race | Indigenous variety | Dark brown seed |
| 23 | IC – 204159 | Land race | Indigenous variety | White seed |
| 24 | IC – 152485 | Land race | Indigenous variety | Medium brown seed |
| 25 | IC – 96230 | Land race | Indigenous variety | Bright black seed |
| 26 | IC – 204063 | Land race | Indigenous variety | White seed |
| 27 | EC – 310448(36) | Exotic variety | Bulgaria | Bright black seed with high micronutrient |
| 28 | EC – 335004(34) | Exotic variety | Bangladesh | Medium brown seed |
| 29 | EC -334973(38) | Exotic variety | Bangladesh | Light brown seed |
| 30 | EC – 335004(34) | Exotic variety | Bangladesh | Medium brown seed |
| 31 | EC – 303431 | Exotic variety | Bangladesh | Light brown seed |
| 32 | EC – 303433(17) | Exotic variety | USA | Medium brown seed |
| 33 | EC – 164966(50) | Exotic variety | USA | Medium brown seed |
| 34 | EC – 164966(52) | Exotic variety | USA | Black seed, early maturing |
| 35 | EC – 310448(39) | Exotic variety | Bulgaria | Black seed |
| 36 | EC – 41923 – (49) | Exotic variety | Bangladesh | Medium brown seed, high iron |
| 37 | EC – 303432 | Exotic variety | USA | Medium brown seed |
| 38 | EC – 303439 | Exotic variety | Bulgaria | Dark brown seed |
| 39 | CUHY-57 | HYV | C.U developed variety | Beige seed ,Hybrid high yielding variety |

| SERIAL NO. | NAME | Local land race/ variety | CENTRE OF ORIGIN | SALIENT FEATURES/ OTHER INFORMATION |
|---|---|---|---|---|
| 40 | CUHY-23 | HYV | C.U developed variety | Beige seed ,Hybrid high yielding variety |
| 41 | CR-11 | HYV | C.U developed variety | Brown seed , Mutant high yielding variety |
| 42 | CUHY-24 | HYV | C.U developed variety | Black seed ,Hybrid high yielding variety |
| 43 | CUMS-17 | HYV | C.U developed variety | Brown seed , High oil content, Mutant high yielding variety |
| 44 | CUHY-13 | HYV | C.U developed variety | Beige seed ,Hybrid high yielding variety |
| 45 | CUHY-36 | HYV | C.U developed variety | Beige seed ,Hybrid high yielding variety |
| 46 | CR-11A | HYV | C.U developed variety | Black seed , Mutant high yielding variety |
| 47 | CUHY-45 | HYV | C.U developed variety | Beige seed ,Hybrid high yielding variety |
| 48 | CUMS-9 | HYV | C.U developed variety | Dark Brown seed , Mutant high yielding variety |
| 49 | CUHY-27 | HYV | C.U developed variety | Beige seed ,Hybrid high yielding variety |
| 50 | CUMS-11 | HYV | C.U developed variety | Brown seed ,Hybrid high yielding variety |

### 2.2 Methods for statistical analysis

Agro-morphological data were recorded for 10 competitive plant for each of the 50 accessions for nine metric traits and the experimental data were subjected to analysis of variance (ANOVA) using the software SPAR2 statistical package and SPSS (16.0).

### 2.2.1AExplanation Of Analysis Of Variance

There are three sources of variability in the design- treatment (variety), replication, and experimental error. At first treatment total, replication totals and grand total was calculated. RBD method was taken from Gomez & Gomez (1983).The outline of ANOVA as follows:-

**Table no: 2.2.1.A.1. Table for outline of ANOVA**

| SOV | DF | SUM OF SQUARE | MEAN SUM OF SQUARE (ss/df) | CALCULATED F VALUE | TABULATE DF VALUE |
|---|---|---|---|---|---|
| Replication | (r-1) | | rss/(r-1) | Rep.ms/error ms | |
| Treatment | (t-1) | | Tss/(t-1) | Tr.ms/error ms | |
| Error | (r-1)(t-1) | | Ess/(r-1)(t-1) | | |
| Total | rt-1 | | | | |

Here,r = no. of replication

t = no. of treatments

CF= (Grand total) $^2$/rt

Total ss = Sum of square of individual observation – CF
Replication ss = (Sum of square of replication/no. of variety) – CF
Treatment ss = (Sum of square of treatment/no. of replication) – CF
Error ss = Total ss – Replication ss – Treatment ss

- **Critical difference**: (CD) - In order to compare the mean of various entries, we require to calculating critical difference (CD).

  CD=$\sigma\sqrt{\sigma}$MSe/r * t at 5% level of significance of error df

- **Coefficient of variation (CV):-** It is the good basis for comparing the extent of variation between different characters with different scales.

  C.V= √MSe/X * 100

**2.2.2.B Explanation for Biometrical Analysis :-**

- **Error variance**: -The expected mean sum of squares for error, E(MSe), i.e$\sigma^2$e, will be purely a random environmental variance. The mean sum of square consists of variances (i) attributable to varietals difference (i.e. genotypic variance) and (ii) due to environmental variation among individuals of each genotype.

  Thus the expected mean sum of square would be

**E (MSv)= $\sigma^2$e + r$\sigma^2$g** **E (MSe)= $\sigma^2$e**

- **Genotypic and Phenotypic Co-Efficient Of Variation**:-

**Genotypicco-efficient of variation (GCV) =** $\sqrt{\sigma^2}$g/X * 100

**Phenotypic co-efficient of variation (PCV)=** $\sqrt{\sigma^2}$p/X * 100

Where X = grand mean of treatments

- **Heritability (Broad Sense):**Heritability denotes the proportion of phenotypic variance that is due to genotype. Here the heritability measured in %.

Thus**, Heritability ($h^2$) = ($\sigma^2g/\sigma^2p$) x 100**

❖ **Genetic Advance**: -It is the improvement of 5% selection intensity in performance of selected lines over the original population

**Genetic Advance (GA) = K x$\sigma$g x ($\sqrt{\sigma^2}g / \sqrt{\sigma^2}p$)**

Where,

K=Selection Intensity which is 2.06 at 5% level

$\sigma$g =Genotypic variance

## 2.3 Methods for Development of Microsatellite Markers

Sesame EST sequences were downloaded in FASTA format from the National Center for Biotechnology Information (NCBI ) data base. (https://www.ncbi.nlm.nih.gov/) as of May 2015. The NCBI database exclusively maintains EST sequences for all plant and animal species. With the help of VecScreen (https://www.ncbi.nlm.nih.gov/tools/vecscreen) we have the removed the vector contamination and adapter, linker and PCR primers contamination. After that we used trimmest (http://embossgui.sourceforge.net/ demo/trimest.html)by which we indentified and removed the poly A tail from the downloaded sequence.

Microsatellites/SSRs were identified using the Simple Sequence Repeat Identification Tool (SSRIT) program (www.gramene.org/db/searches/ssrtool) Simple scripts (written in Visual Basic) were then used to parse SSRIT output into the relational database. EST sequences less than 200 bp were removed. SSR-containing EST sequences were clustered using Cap3 program to identify the non-redundant EST sequences The Cap3 algorithm computes overlaps between sequences and then joins the reads in decreasing order of overlaps to form contigs.

Non-redundant SSR containing EST sequences of sesame were used for homology search using the Basic Local Alignment Search Tool (BLAST) available in the NCBI database (http://www.gramene.org/db/searches/blast). Among all the BLAST hits identified for a particular sesame EST, one having the maximum score was selected. This was followed by identification of homologous genomic region on sesame chromosome. BLAST searches were performed to provide complete coverage across the sesame genome sequence. For this purpose, each sesame chromosome was divided arbitrarily into five equal parts and named as “top”, “middle”, “bottom”, “region between top and middle” and “region between middle and bottom”. The top scoring ten SSR containing sesame EST sequences for each region of each sesame chromosome were picked. Thus a total of 235 SSR-containing non-redundant sesame EST sequences were selected. These sesame SSR-containing EST sequences were expected to sparsely cover the entire nuclear genome of rice, and thereby similarity covers the corresponding sesame genomic regions.

Primer pairs were designed for selected 235 non-redundant SSR-containing sesame EST sequences using Primer3 program after masking the repeat units (http://biotools.umassmed.edu/bioapps/primer3_www.cgi). The primer pairs were designed to meet the following conditions: primer length (min-18nt, opt-22nt, max- 24nt), Tm (min-54˚C, opt-57˚C, max-60˚C) and GC content (min-45%, opt-50%, max- 60%).

50 sesame genotypes were collected from diverse eco-geographical regions of the world. These genotypes were cultivated in Kharif-15 season following Randomized Block Design (RBD) with 3 replications having 10cm spacing between plants and 40cm between rows. The experiment was carried out at Agricultural Experimental Farm, University of Calcutta, Baruipur during Rabi-summer season, 2015. The farm is situated at an elevation of 10 meter above sea level, at approximately $22^0 51^/$ N latitude and $88^0\ 24^/$ E longitude.

**2.3.1 DNA extraction**

DNA extraction was mainly carried out from young leaves of sesame plants around 8–12 days old (2-4 leaves stage). Extraction of DNA was done as described by Saghai-maroof et al. (1984). The purified DNA was quantified with Nanodrop Lite (Thermo Scientific, USA).

**2.3.2 Est-SSR analysis**

235Est-SSR microsatellite marker were selected for the study. Each 25µl reaction mixture containing 0.2 µmolL−1 SSR primers, 0.2 mM of each dNTPs, 2 mmolL−1 MgCl2, 1 X PCR buffer and 0.5 unit Taq polymerase (Life Technologies, New York, USA) and 50 ng sample DNA. The program for SSR and Est-SSR was carried out in a DNA thermo cycler (model-Pro, Eppendorf AG 6321, Germany). The program was set up as follows: denaturation at 95°C for 5 min, 40 cycles 2 min at 95 °C, 45 s at on annealing temperature of the primer, 72 °C for 30 second with final extension at 72 °C for 5 min. Amplified PCR products were separated on 2 % agarose gel (Sigma USA) stained with ethidium bromide (Biorad, USA). A 50 base pair ladder marker (Gene Ruler 50 bp DNA Ladder, Thermo Scientific, USA) was used to estimate PCR fragment size.

**2.3.3 Genetic diversity analysis using ssr profiles**

The analysis was done with the help of 1-0 matrix. Those band were selected which was clear and visible for data analysis. Presence or absence of bands related to each primer was scored as '1' or '0' respectively. PIC was calculated by PIC = 1-∑ (Pi)2, where Pi is the proportion of samples carrying the ith allele of a particular locus) .Effective allele per locus (Aep) was computed as suggested by Weir (1990) by 1/(1-Hep), where Hep designates the genetic diversity for each locus and Hep is equal to 1-ΣP2i, Pi means the frequency of ith allele at the locus. Expected heterozygosities (HE) calculated with the help of the formula [N(N+1)] / 2 Where n is number of alleles. All the data were analyzed for multivariate using the software Darwin Ver 6.0 (Sneath and sokol, 1973). The data were first standardized to eliminate the effects of different measurement following STAND option. The distance coefficient utilizing DICE similarity index

was then worked out by utilizing the transformed data and the information was epitomized in dendrogram following unweighted pair group method with arithmetic average (UPGMA), and Shan clustering program in Darwin Ver. 6.0 The un-weighted neighbour joining (NJ) method as implemented in DARwin 6.0 software was used to generate dendrogram using the simple-matching dissimilarity matrix to determine the aggregation of the accessions into clusters. Un-weighted neighbour joining gives a same unitary weight to all units.

### 2.4 Methods for Association Mapping

#### 2.4.1 Selection of accessions and determination of phenotypic data

A total of 50 sesame accessions were selected for genotype screening and evaluation to identify loci associated with yield components and oil content QTLs. All of the collections were derived from four sources: 1) local and landrace varieties in West Bengal, India; 2) indigenous with outstanding yield components or protein and oil content. 3) Parental lines that are typically used in breeding programs 4) and historical varieties and germplasm resources lines from abroad, including four collections from the USA, three from the Bulgaria, five from the Bangladesh.

#### 2.4.1 Data analysis:

LD values ($r^2$ and p value) between marker fragments were calculated using TASSEL 2.0 software (Kodad et al;2011). The genetic distances between marker pairs were calculated based on the position of these markers on the genetic map (Font I Forcada et al ;2013, Ganopolus et al;2011). Minor loci with a frequency < 0.05 were filtered out to reduce problematic and biased LD estimations between pairs of loci (Kodad et al ,2006, Krill et al;2010). The $r^2$ values for pairs of SSR loci were plotted as a function of map distances, and LD decay ($r^2 < 0.1$) was estimated using the average distances of marker pairs showing LD values lower than 0.1 (Law ,M.2000).

The population structure was analyzed using STURCTURE 2.2 software (Lindsey et al 2003), with a running time of 100,000 and 100,000 replications after burn-in. Models for admixture and correlated allele frequencies were employed in the population structure analysis. The pair wise kinship of all collections was calculated using TASSEL 2.0 software (Kodad et al;2011). The MLM association analysis of the yield components and oil content traits was performed with TASSEL 2.0 software, incorporating filtered marker data and the K and Q matrices. We also performed GLM association analyses using the same four datasets, incorporating pair wise kinship information as a covariate and 1,000 permutations for the correction of multiple testing. To make up for the deficiency of using p-values in association, significant MLM associations ($p < 0.05$) across more than two environments were ranked, and the significance of these markers ($p < 0.05$) in the permutation test was compared using GLM association tests. The p-values derived from the MLM and GLM analyses were also separately tested using the positive false discovery rate (pFDR) test (Martinez-Gomez et al 2003) for multiple testing corrections. The minimum Bayes factor (BFmin) was calculated using following formula: BFmin = -e*p*ln(p) (Fernandez I Matri et al 2009, Merah et al 2012, Mnejja et al 2010).

## 3. Results and Discussion:

## 3.1 Character association among yield and yield components

Yield is a very complex quantitative character and is also affected by environmental fluctuation. The knowledge about yield and yield components help to achieve the desired level of improvement in yield.

## 3.1.1 ANALYSIS OF VARIANCE (ANOVA)

ANOVA for nine characters revealed that replications differed much for most of the characters (Table-**3.1.1.1**). Treatments were significantly different from each other indicating high diversity.

## 3.1.2 Estimation of genetic parameters:

Characterization of genotypes would be helpful for breeders to utilize appropriate characters in sesame improvement programs. Agro-morphological characterization is one of the most useful tools for the plant breeders for identifying a suitable parent/ genotypes for future breeding improvement. It is focused on the yield and yield attributing traits which have a gross effect on improvement of grain yield. The traits which selected for the analysis are affected by the environment. Genotypic coefficient of variation (GCV) measures the variability of any character and phenotypic coefficient of variation PCV indicated substantial role of environment in expression of the characters. Heritability indicates the magnitude of transmission of a character from parents to offspring and it is used as predictive role in selection method (Allard, 1960). It gives an idea of the total variation ascribe to genotypic effects, which are exploitable portion of variation (Akinwal et.al. 2011). Heritability serves as a useful guide for the breeding program. If the heritability of a character is very high *e.g.*, 60% or more selection for the character should be fairly easy because of a close correspondence between the genotype and the phenotype due to a smaller contribution of the environment to the phenotype. But if heritability low i.e less than 40% for a character selection may be considerably difficult or nearly impractical due to the masking effect of the environment on genotypic effect. According to Johnson *et al.*(1955) the heritability is considered to be high if it is more than 60%. In addition to the broad sense heritability, genetic advance helped to predict the genetic gain to the next generations. The extent of the environmental influence on any character is indicated by the magnitude of the differences between the genotypic and phenotypic coefficients of variation. Large differences reflect high environmental influence, while small differences reveal high genetic influence.

The selection of desirable traits is based on the high genotypic co-efficient (GCV) combined with high heritability and genetic advance and because those traits are governed by additive gene action.

The estimates of genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) exhibited that PCV was greater than GCV for all traits indicating substantial role of environmental effect in the character expression. ((Table no: 2.6.2.1, 2.6.2.2 and 2.6.2.3)

Similar finding were reported by Iqbal *et al.*, (2016 & 2015), Begum and Dasgupta (2014), Tripathi *et al*., (2013) and Saha *et al.*,(2012) in sesame.

Highest PCV was recorded for seed yield per plant (48.74) followed by capsules per plant (48.18), number primary branches/plant (44.55), iron content (35.57) , 1000 seeds weight (23.61), inter node length (23.09). Conversely, moderate to low PCV was observed for the traits, plant height, capsule length, days to 50% flowering, capsule breadth and days to maturity (Table **3.1.2.1** ). The broad sense heritability estimates were found to be higher for 1000 seed weight (92.47%), iron content (83.8%), zinc content (81.79 %), number of capsules per plant (76.37%) , seed yield/plant (74.44%), number primary branches/plant (66.87), capsule length (64.28%), plant height (63.64%), while days to flowering (58.26%), capsule breadth (34.58%) days to maturity (31.99%) and internode length ( 29.09 %) exhibited moderate to low heritability. High GA % of mean was observed for characters like number of capsules per plant (56.92), seed yield/plant (53.11) number primary branches/plant (33.33) and seed weight (29.44).

**Table: 3.1.1.1 Analysis of variance (ANOVA) for the yield and yield attributing traits with iron, zinc and protein content**

| Source | DF | Plant height | Days to 50% flowering | Days to maturity | No. Of primary branches/plant | Capsule length | Capsule breath | No. Of capsules/plant | 1000 seed weight | Seed yield/ plant | Iron content | Zinc content |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Replications** | 2 | 253 | 11.37** | 5.31** | 3.44 | 0.01 | 0.40 | 497.31** | 0.29 | 0.21 | 1.76 | 0.52 |
| **Treatments** | 69 | 779.41** | 16.68** | 31.04** | 23.35** | 0.45 | 0.006 | 2833.52** | 0.36 | 41.74** | 4532.6** | 186.9** |
| **Error** | 138 | 86.68 | 4.58 | 5.43 | 2.14 | 0.029 | 0.003 | 309.145 | 0.031 | 2.09 | 1.56 | 0.13 |

*Significant at 5% level of significances at error d.f.
**Significant at 1% level of significances at error d.f.

**Table: 3.1.2.1 Estimates of genetic parameters (2015)**

| | G.C.V (%) | P.C.V (%) | H (%) | GA | GA % of Mean |
|---|---|---|---|---|---|
| **Plant height** | 18.1 | 20.82 | 63.64 | 35.6 | 21.1 |
| **Days to 50% flowering** | 8.6 | 11.26 | 58.26 | 6.32 | 5.15 |
| **Days to maturity** | 3.53 | 6.23 | 31.99 | 1.09 | 0.84 |
| **No. Of primary branches/plant** | 41.12 | 44.55 | 66.87 | 2.98 | 33.33 |
| **Inter node length** | 12.41 | 23.09 | 29.09 | 0.6 | 10.88 |
| **No. Of capsules/plant** | 43.13 | 48.18 | 76.37 | 63.033 | 56.92 |
| **Capsule length** | 13.1 | 16.35 | 64.28 | 0.38 | 12.54 |
| **Capsule breadth** | 8.23 | 13.99 | 34.58 | 0..29 | 8.55 |
| **1000 seed weight** | 22.70 | 23.61 | 92.47 | 1.10 | 29.44 |
| **Seed yield/ plant** | 42.05 | 48.74 | 74.44 | 5.41 | 53.11 |
| **Iron** | 34.55 | 35.57 | 83.8 | 30.37 | 28.73 |
| **Zinc** | 13.5 | 14.89 | 81.79 | 17.84 | 28.28 |

## 3.1.3 Correlation Coefficient:

The magnitude of correlation among the traits is the key factor while the complex trait like yield is under consideration. Steel and Torrie (1984) stated that correlations identify the measurement of the intensity of association between the traits. Further, the study helps the breeder to understand the mutual component characters on which selection can be based for genetic improvement. In general, phenotypic and genotypic correlation coefficients are closely related each other. Genotypic correlations were higher in magnitude which elucidate that when the genes governing two traits were similar and modifying/masking the effect of environment in the character expression.

Significant and positive correlation was observed between Fe and Zn (Table 3.1.3.1) suggested that simultaneously component in likely to be highly effective. Highly significant correlation between Fe and Zn was earlier by (diapari *et al*, 2014) in chickpea. There was no correlation between seed yield and Fe and Zn contents in both the population indicating simultaneous selection foe Fe and Zn can be accomplished without compromising on seed yield. With Fe and Zn content in both the population indicated breeding for higher level of micronutrients without compromising the improvement for large grain size. And Zinc negatively correlated with days to 50% flowering, days to maturity and seed yield per plant. Highly significant positive phenotypic correlation coefficients was observed between seed yield/plant and plant height, capsules/plant and 1000 seed weight The results agreed with earlier Works by Sumathi and Murlidharan (2010). Khan *et al*., (2001), Uzun and Cagirgan (2001) and Sumathi *et al*., (2007) and Subramanian & Subramanian (1990). This emphasized that selection for higher Fe content would like to improve Zn also through correlated response in positive direction and 1000 seed weight in negative direction. The present findings of highly positive correlation between Fe and Zn find support Morgounov *et al*., 2007 in wheat. Correlation coefficient analysis is an important estimate in the determination of most effective statistics. Component breeding becomes simple task when there is a positive association of important characters. But when the characters are negatively associated, it would be difficult to exercise simultaneous selection for them in restructuring a variety. It is important to improve the micro-elements along with augmenting productive of crops. Some of the nutrients like Fe and Zn, obviously had a good impact on human nutrition. In fact both the elements to be positively associated and simply by conventional breeding program, it is possible to Fe and Zn content in seeds following simple breeding program. Among the morphological traits, seed yield was correlated positively with number of capsules/plant. Thus it is possible to increase seed yield further by augmenting this trait. Interestingly, Fe and Zn content were not correlated to either of the traits, thus restricting of plant type with high yield with higher content of Fe and Zn are achievable targets through simple breeding approach like transfer of gene for high Fe and Zn content to high yielding genotypes.

Table 3.1.3.1 **Correlation coefficient for micronutrient content with yield and yield related traits**

| | PH | PB | SB | DF | IL | DM | CP | CL | CB | SW | SYP | Fe | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PH | 1 | | | | | | | | | | | | |
| PB | .392* | 1 | | | | | | | | | | | |
| SB | -.106 | .107 | 1 | | | | | | | | | | |
| DF | .217 | .121 | -.004 | 1 | | | | | | | | | |
| IL | .266* | .389** | -.263* | .027 | 1 | | | | | | | | |
| DM | .150 | .210 | .168 | .015 | .182 | 1 | | | | | | | |
| CP | .469** | .190 | .062 | -.033 | -.080 | .043 | 1 | | | | | | |
| CL | -.148 | -.046 | -.225 | -.116 | -.016 | .061 | -.186 | 1 | | | | | |
| CB | .080 | .267* | -.198 | .322* | .091 | -.034 | -.129 | .292* | 1 | | | | |
| SW | .197 | .523** | -.142 | .038 | .214 | .195 | .194 | .061 | .355* | 1 | | | |
| SYP | .425** | .124 | .077 | .146 | -.067 | -.206 | .330* | -.119 | .159 | .248* | 1 | | |
| Fe | .195 | -.020 | -.185 | .192 | -.029 | .008 | .055 | -.322* | .154 | .057 | .173 | 1 | |
| Zn | .214 | .012 | .057 | -.031 | .076 | -.001 | .044 | -.141 | .085 | 0.47 | .126 | .267* | 1 |

PH= Plant height, PB= Primary Branches, SB= Secondary branches, DF = 50% flowering, IL= inter node length, DM = Days to maturity, CP= Capsule/ plant, CL = Capsule length, CB= Capsule breath, SW= 1000 seed wt, SYP = Seed yield per plant, Fe= iron, Zn= Zinc

### 3.1.4 Path Analysis:

In order to have more closer view of relationship between traits it is imperative to provide an effective means of untangling direct and indirect causes of association which would permit a critical examination of the specific forces acting to produce a given correlation. An attempt has been made in the present study to find out the direct effect of each causal factor component and its indirect effect via another factor component on seed yield/plant with the help of path coefficient analysis (Wright 1934). This method has already been proved to be very useful in plant selection and breeding depending upon one or more causal factor (Dewey and Lu, 1959). (Mohammad et al, 2003; Williams et al, 1990; Moghaddan et al 1998;)

A path coefficients is a standardized partial regression coefficient and measures the direct influence of a predictor variable on the response variable (Li, 1975) result showed that the most important agronomic trait determining seed yield in the path coefficient analysis were plant height exhibited highest positive direct effect on seed yield/plant (Table-**3.1.4.1** ) similar result is reported by Sumathi et al (2010), and Khan et al ( 2001) the other important contributing characters are, , no of secondary branches per plant , no. of capsules/plant, capsule length , capsule breadth and 1000 seed weight. The indirect effects of no. of capsules/plant, number of seeds/capsule and 1000 seed weight were also positive on seed yield via several other characters. In spite of the .height .positive direct effect among seeds yield and the mentioned traits. Days to 50% flowering, no of primary branches per plant and Days to maturity had a negative direct effect Sumathi et al (2010). According the results of our study , it can be concluded that to increase seed yield, selection should be carried out for higher biological yield, harvest index ,1000 seed weight , number of capsule in main stem and lower height of the first capsule from thebe soil .surface. Also, selections .for high oil content and stem diameter are contrary to achieve potential yield.

**Table 3.1.4.1 Path coefficient analysis of twelve characters on seed yield per plant at phenotypic level**

| | Plant height | Primary Branches | Secondary branches | 50% flowering | Inter node | Days to maturity | Capsule /plant | Capsule length | Capsule breath | 1000 seed wt | Fe | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Plant height | **.586** | .230 | -.062 | .127 | .156 | .088 | .275 | -.086 | .046 | .115 | .115 | .125 |
| Primary Branches | -.150 | **-.383** | -.041 | -.046 | -.149 | -.080 | -.072 | .017 | -.102 | -.200 | -.200 | -.004 |
| Secondary branches | -.040 | .041 | **.386** | -.001 | -.101 | .064 | .023 | -.086 | -.076 | -.054 | -.054 | .022 |
| 50% flowering | -.002 | -.001 | .000 | **-.012** | -.000 | -.000 | .000 | .001 | -.004 | -.000 | -.004 | .001 |
| Inter node | -.000 | -.000 | .000 | -.000 | **-.000** | -.000 | .000 | .000 | -.000 | -.000 | -.001 | -.001 |
| Days to maturity | -.058 | -.081 | -.065 | -.005 | -.070 | **-.388** | -.016 | -.023 | .013 | -.075 | -.075 | .000 |
| Capsule/plant | .013 | .005 | .001 | -.000 | -.002 | .001 | **.028** | -.005 | -.003 | .005 | .005 | .001 |
| Capsule length | -.000 | -.000 | -.000 | -.000 | -.000 | .000 | -.000 | **.003** | .001 | .000 | .001 | -.001 |
| Capsule breath | .007 | .023 | -.017 | .028 | .008 | -.003 | -.011 | .025 | **.088** | .031 | .031 | .007 |
| 1000seed wt | .112 | .297 | -.080 | .021 | .121 | .110 | .110 | .034 | .201 | **.568** | .007 | .267 |
| **Fe** | .026 | -.002 | -.025 | .026 | -.003 | .001 | .007 | -.044 | .021 | .007 | **.568** | .024 |
| **Zn** | -.067 | -.003 | -.018 | .009 | -.024 | .001 | -.013 | .044 | -.027 | -.149 | -.149 | **-.317** |

**Residual effect = 0.236**

**Bold figures are direct effects**

### 3.1.5 Principal Component Analysis

Principal Component Analysis measures the importance and contribution of each component to total variance. It can be used for measurement of independent impact of a particular trait to the total variance whereas each coefficient of proper vectors indicates the degree of contribution of every original variable with which each principal component is associated. In present investigation, Principal component analysis has shown the genetic diversity of 50 genotypes following the Proportion of Variance Criterion by O'Rourke and Hatcher. According to this criterion, four principal components with cumulative variance of 66.93% (Table no: **Table 3.1.5.1**). which will provide a prominent idea of structure underlying the variables analyzed. For this study the first four axes obtained with Eigen value of > 1.0; which indicates that the identified traits within the axes exhibited great influence on the phenotype of the germplasm set. PCA1 accounted for 19.321 % of total variation in the population having the contribution from pH followed by Capsule per Plant, Seed Yield per Plant, 1000 Seed Weight and Zinc. Hence, the first component has phenological and yield related variables similar finding was reported by Sanni *et al.* (2008).

Second Principal component accounts for 15.013% of total variation having major contribution of the traits like iron, zinc, Capsule per Plant and Seed Yield per Plant. Hence, the second component has micronutrient and agronomic traits. PCA 3 was accounted for 12.81% of total variation and the traits contribute most are capsule length, capsule breadth, primary branches and 1000 seed weight. PCA 5 was accounted for Days to 50 % Flowering, Days to Maturity and Capsule per Branch. PCA 4 was accounted for 10.213% of total variation and the having more contribution of the characters like Primary Branches, Secondary Branches and Days to 50% flowering. Hence, from the current investigation the five three principal components contributing about half of the variance were plotted to observe relationships between the measured traits. The scree plots (Figure. 3.1.5.1 & 3.1.5.2) of PCA describe for first four eigen values and correspond to the whole percentage of the variance observed in the dataset revealed the high level of genetic variation existing in the population panel and explains the traits contributing for this diversity. Hence the results will be of greater benefit to identify parents for improving various morphological traits analyzed in this study. The PCA may be deemed important if their associated coefficients are of relative magnitude with breeding targets and given this apparent potential for using PCA, further work is required to compare multivariate methods for reaching actual gains.

**Table 3.1.5.1 Eigen value and percent of total variation and component matrix for the principal component axes**

| Parameter | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|
| PH | .707 | -.216 | -.026 | .006 | .083 |
| CP | .694 | .526 | -.218 | .236 | .016 |
| SYP | .682 | .596 | .033 | .172 | .095 |
| SW | .473 | .084 | .464 | -.130 | .213 |
| DM | -.432 | .086 | .231 | .255 | .427 |
| ZN | .470 | -.695 | -.080 | .110 | .182 |
| FE | .107 | -.595 | -.238 | .377 | .406 |
| CL | -.041 | .032 | .676 | -.267 | .274 |
| CB | .086 | -.118 | .638 | .245 | -.501 |
| PB | -.204 | .366 | -.419 | -.415 | .308 |
| SB | .301 | -.199 | .200 | -.637 | .250 |
| DF | -.295 | .288 | .278 | .444 | .470 |
| Eigen Value | 2.319 | 1.802 | 1.538 | 1.228 | 1.146 |
| Variability (%) | 19.321 | 15.013 | 12.817 | 10.231 | 9.551 |
| Cumulative (%) | 19.321 | 34.33 | 47.151 | 57.382 | 66.934 |

PH= Plant height, PB= Primary Branches, SB= Secondary branches, DF = 50% flowering, IL= inter node length, DM = Days to maturity, CP= Capsule/ plant, CL = Capsule length, CB= Capsule breath, SW= 1000 seed wt, SYP = Seed yield per plant, Fe= iron, Zn= Zinc

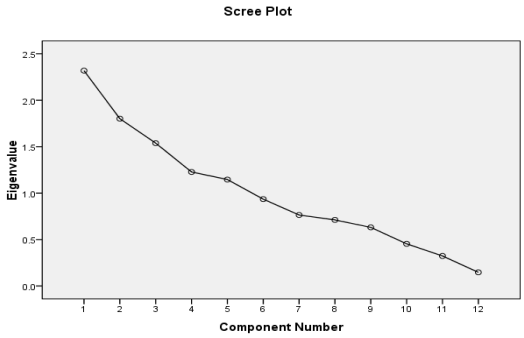



**Figure 3.1.5.1. Scree Plot**

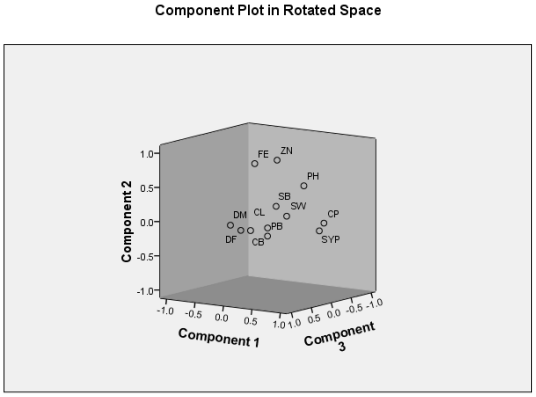



**Figure 3.1.5.2. Component plot in rotated space**

### 3.2. EST-SSR development:

All 44010 EST sequences available for sesame in the NCBI database were downloaded in FASTA format. EST sequences of more than 200 bp only were considered for identification of SSR using SSRIT. A total of 9,106 sesame EST sequences were found to contain microsatellites. Among SSR containing EST sequences, only 7.9% belonged to Class I (motif length ≥ 20 nucleotides) and remaining were belonged to class II type (motif length < 20 nucleotides).

Clustering of SSR-containing EST sequences using Cap3 program eliminated redundancy in the dataset: 9106 sesame EST sequences (redundant) were reduced to 2184 non redundant EST sequences or unigene sequences that contain microsatellites motifs. In total, In total, only about 4.9 % of sesame EST sequences were found with microsatellite motifs. only about 23.98% of non-redundant sesame EST sequences are containing microsatellite motifs The AG class of di-nucleotide repeats which includes CT, GA and TC repeats and the CCG class of tri-nucleotide repeats which includes CCG, GGC, CGC, GCC, GCG and CGG were the most abundant in sesame. The frequencies of tri-nucleotide repeats belonging to ATT (Isoleucine) and AAT (Asparagine) classes were very low (0.57% and 0.27%, respectively).

Rice-sesame synteny was exploited for selection of a sub-set of non-redundant SSR containing sesame EST sequences. Sesame EST sequences were BLAST searched against the rice genome in the GRAMENE database to get top ten hits on each of the five arbitrary regions of all rice chromosomes

To identify these 235 candidate sequences from 2184 non-redundant sesame ESTs containing SSR sequences, 1483 EST sequences (14.76%) were BLAST searched against the rice genome to obtain uniform and complete coverage on rice with the expectation that this would similarly provide uniform and complete coverage across the sorghum genome.

The list of 300 putative sesame EST containing SSR sequences selected based on BLAST. This contains the information on sesame EST identity, BLAST score, on which chromosome of rice having the hit.

Among the selected 300 candidate sequences, 241 sequences (80.3%) had putative annotations. The majority of these are classified as transcription factors or DNA binding proteins. Among the 300 selected sesame ESTs containing SSRs, 33 were di-nucleotide repeats (11%); 205 were tri-nucleotide repeats (68.3%); 38 were tetranucleotide repeats (12.66%) and 24 were penta-nucleotide repeats (8%). The most abundant repeats among the selected EST sequences were AG and CCG classes as found across the full set of 2184 non-redundant sorghum EST sequences containing SSRs. A total of 39 of these EST-SSRs (13%) were Class I and 236 EST-SSRs were Class II, while the remaining 25 EST-SSRs were only 10 nucleotides (five di-nucleotide units) in length.

For the selected 300 candidate SSR-containing non-redundant sesame EST sequences, primer pairs were designed using Primer3 program after masking the repeat motif. These primers were named as CALCUTTA UNVIVERSITY EST SSR Primer (CU-ESSR) pairs with three digit serial number.

Seventy diverse genotypes collected from different part of the world were screened with all designed primer pairs. Out of 235 primer pairs, 157 (66.8%) amplified the template DNA and 126 (80.2%) of them produced simple and easy to score amplification products, whereas the remaining 19.8% produced multiple fragments that were difficult to score. Most of the primer pairs that produced no amplification or gave non-specific amplification targeted tri-nucleotide repeats. Very few primer pairs gave the amplicon sizes more than 500 bp while most of the primer pairs gave the amplicons sizes in the range of 150-200 bp.

Polymorphism between the parents involved was scored simultaneously with PCR optimization. Of the 157 primer pairs that produced good amplification profiles, 35primer pairs (22%) detected polymorphism in diverse collected genotypes of sesame.

The present investigation distinctly delineated that primers were efficient enough to distinguish the sesame genotypes in (Table 1) summarizes the result based on the analysis of the 50 sesame genotypes using the polymorphic ESR-SSR loci. The number of alleles varied among these loci. Among the 235 markers used in the analysis, 35 (15%) showed polymorphism with a total of 151 alleles identified across genotypes with an average of 4.34 alleles per locus, with sizes from 68 to 666 bp. The lowest band was observed in CU-ESSR-10 whereas highest band size was observed in the CU-ESSR-11. The number of alleles ranged from 3 ( CU-ESSR- 09, CU-ESSR- 14, CU-ESSR- 15, CU-ESSR- 20, CU-ESSR- 25 , CU-ESSR- 30, CU-ESSR- 32) to 6 (CU-ESSR-02, CU-ESSR- 06, CU-ESSR- 28, CU-SSR- 33. On a per locus basis, these numbers were much higher than the average 2.0-5.5 alleles per locus for various classes of microsatellites reported by Cho *et al.,* (2000) and Yu *et al.,* (2003), using different parental lines for an international molecular breeding programme. The mean allele number found in this study, 4.34, was slightly lower than 7.6 obtained by Cho *et al.,* (2011), higher than 3.11 reported by Badri *et al.,* (2014) but very similar to 4.7 obtained by Dixit et al 2005 in sesame. One possible reason for this discrepancy in the mean number of alleles (7.6 vs. 4.34) could be the inclusion of wild genotypes by Cho *et al.,* (2011) resulting in more alleles, some of which novel, whereas only domesticated genotypes was used in the present study, possibly having narrowed their genetic base.

It was revealed from the molecular data (Table **3.2.1** ) that the EST-SSR primers CU-ESSR- 14 followed by CU-ESSR- 12 and CU-ESSR- 27 and CU-ESSR-28 respectively were more polymorphic while, SSR loci CU-ESSR- 18) showed a minimum of polymorphisms. Variable allelic diversity in microsatellite markers have been reported by several scientists (Akagi *et al.,*1997, McCouch*et al.,* 2002) concluded that microsatellite markers demonstrate high genetic diversity per locus because of their multi-allelism.

The polymorphic information content (PIC) ranged from 0.36 (CU-ESSR-18) to 0.82 (CU-ESSR-26, Table**.1**) with an average of 0.607. This value of PIC is slightly lower than 0.72 which was reported by Yepuri et al., (2013). But much higher than the PIC value reported by Cho et al. 2011 and Lin-Bin et al., 2008 who reported 0.42 and 0.39 respectively. Based on PIC values,CU-ESSR-18 (0.36) appears to be the least informative followed by CU-ESSR-16 (0.41), whereas, CU-ESSR-26 (0.73) would appear to be the most informative.

The PIC values indicate the highly informative nature of these microsatellites. The newly isolated microsatellite markers are expected to provide a valuable resource for diversity analysis among a large germplasm collection of sesame. Comparison of molecular marker information indicates that the genetic basis was narrowed down and the genetic diversity was declining during domestication and selection of mordent cultivars from landraces.

In the present study, sesame –specific microsatellite markers has been developed by the mining of EST data base. Dixit at el. (2005) reported the ten SSR markers in sesame. This report was the initial but after 2005 not such report of SSR is came till 2011. To cover the entire genome, numbers of microsatellite markers are still minimal.

## 3.3 EST-SSR Analysis of the Accessions

Amplification of the 35 EST- SSR loci was successful in all the sesame genotypes, producing well- defined and reproducible bands. The primers produced a total of 151 different alleles, with an average of 4.34 alleles per locus.

Seven markers namely4S35384247, 4S37067373, 4S37067501, 4S39975317, 4S45378466, 4S60767943, 4S63721981 showed only three alleles whereas four markers namely 4S29209539, 4S33862684, 4S49713883, 4S60768111 produced 6 different alleles (Table 5.4.1.1). The mean allele number found in this study, 4.34, was lower than 7.6 obtained by Cho et al. (2011), but very similar to Dixit et al 2005 in sesame. One possible reason for this discrepancy in the mean number of alleles (7.6 vs. 4.34) could be the inclusion of wild genotypes by Cho et al. (2011), resulting in more alleles, some of which novel. On the contrary only domesticated germplasm involving high yield varieties and landraces was used in the present study, possibly having narrowed their genetic base. All primers produced at least of one bands per genotype. The observed heterozygosity ranged from 0.36 (4S38941090) to 0.82 (4S45378492), with an average of 0.61 across all 35 EST- SSRs (Table 5.4.1.1). Ho and He values were compared with the fixation index (Fis) which was on the average 0.12, ranging from −0.29 (4S32099732) to 0.46 (4S38941090). The high F-values observed corresponding to high homozygosity, particularly in individuals with only one band, suggests the presence of null alleles (Brookfield, 1996). The F-value was positive in 36 and negative in 4 EST- SSR loci, thus indicating the high level of heterozygosis in the cultivars studied, as it would be expected in an sesame.

The average heterozygosity value of 0.71 is similar to Dixit et al (2005), but slightly higher than other values reported in sesame, 0.46 by Cho et al. (2011) and 0.21 reported by Zhang et al and 0.54 obtained by Chapman el al.(2009) in safflower.

Table 3.3.1 : genetic parameters of 50 sesame genotypes based on 35 EST-SSR loci

| SR no. | Accesions | Primer sequence | Number of allele | Effective allel (Aep) | PIC value | $H_E$ | Fis |
|---|---|---|---|---|---|---|---|
| **CU-ESSR -01** | **4S29209351** | **F: AGGAAGTGGTGTAACTGTTGA R: TCTTCTCTTCCTCAGTCTCCT** | 5 | 4.35 | 0.46 | 0.77 | 0.273 |
| **CU-ESSR -02** | **4S29209539** | **F:AAGAAAGCTAAGAAGGCAGAG R:GCTTGATAGAGAAGTTACGACA** | 6 | 5 | 0.41 | 0.80 | 0.113 |
| **CU-ESSR -03** | **4S32099560** | **F: TTTATTTATTACCCTCTCCTCT R: CTGAACAACAAACAAAGAAGG** | 4 | 2.94 | 0.54 | 0.66 | 0.182 |
| **CU-ESSR -04** | **4S32099732** | **F: AGCCAGATAAGTTTAGCATGA R: ATTCATTCATTCACTGCTGTT** | 4 | 3.33 | 0.62 | 0.70 | -0.129 |
| **CU-ESSR -05** | **4S33860915** | **F: CCATACACATCCGACGTATTA R: CAACTCCAGCATCTACAACTC** | 4 | 3.33 | 0.69 | 0.70 | -0.014 |
| **CU-ESSR -06** | **4S33862684** | **F: TGTTATACTCAGCCAGTCACC R:TGGTTGGGTTGATATAGTAGG** | 6 | 5.26 | 0.51 | 0.81 | 0.370 |
| **CU-ESSR -07** | **4S35051574** | **F: AATTACCCACAAAAAGAATCC F: AATTACCCACAAAAAGAATCC** | 5 | 3.57 | 0.60 | 0.72 | 0.167 |
| **CUE-SSR -08** | **4S35051622** | **F: ACTCTCCTCTTCAACCTTCAC R: GAAGAGGTGGAGGAATTACG** | 5 | 4.76 | 0.53 | 0.79 | 0.329 |
| **CU-ESSR -09** | **4S35384247** | **F: GATTGTTGAAGAAGAAGGTGA R: TGGCTGAATCTTGAAAATCTA** | 3 | 2.70 | 0.67 | 0.63 | -0.063 |
| **CU-ESSR -10** | **4S35769506** | **F: ACGAGAAAAATGGTTGTGTAA R: TTTACTGGTGTGTGTGTGTGT** | 5 | 4.76 | 0.56 | 0.79 | 0.291 |
| **CU-ESSR -11** | **4S35769858** | **F: ATGCAAAAATACACACACACA R: CGCCACATTTTATGCTTATT** | 5 | 3.57 | 0.57 | 0.72 | 0.208 |
| **CU-ESSR -12** | **4S36047409** | **F: TTGTCAAAGTCAAGAGTTCGT R: TCTTATCCTTGCTAACAGCAG** | 4 | 3.45 | 0.66 | 0.71 | 0.070 |
| **CU-ESSR -13** | **4S36047549** | **F: GCAAAGGTAGAATTGAACAAG R: ATTAGCTTTCTTCAACCCTCT** | 4 | 3.57 | 0.6 | 0.72 | 0.167 |
| **CU-ESSR -14** | **4S37067373** | **F: CCCGGCTTTTCTTCTACTACT R: GGTTGTAGGTGTTGTTGTAGG** | 3 | 2.56 | 0.79 | 0.61 | -0.113 |
| **CU-ESSR -15** | **4S37067501** | **F: CGGTAAGATGACAAACAAGTC R: ATAGTCACCTGTTTTGAATGC** | 3 | 3.03 | 0.57 | 0.67 | 0.149 |
| **CU-ESSR -16** | **4S37185517** | **F: GAAGCCGTTGAATAGAAGAAT R: TACAGCAATGATGAAACAACA** | 5 | 3.57 | 0.43 | 0.72 | 0.403 |
| **CU-ESSR -17** | **4S38941049** | **F: CTACTGCACATTTCCTCATTC R: AGAAATATGATCCCCACTAGC** | 5 | 3.70 | 0.53 | 0.73 | 0.274 |
| **CU-ESSR -18** | **4S38941090** | **F: TGGAAAATTAACTCACAAAGG R: GAATGAGGAAATGTGCAGTAG** | 5 | 1.64 | 0.36 | 0.67 | 0.463 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **CU-ESSR-19** | **4S39975220** | **F:GGGGCTCATAATTCTCTTTT R:CTGTGGGCAATAACAGTAAGA** | 4 | 3.45 | 0.66 | 0.72 | 0.083 |
| **CU-ESSR-20** | **4S39975317** | **F:TCCTCTTTCACTCTCTTTTCC R: CATAAGAGCAAACAGGATGAG** | 3 | 3.82 | 0.72 | 0.77 | 0.065 |
| **CU-ESSR-21** | **4S41178032** | **F:GGGGACTCTTCTTCTTCTTCT R: GAGGACCGTTGTAGTATCCTT** | 4 | 3.57 | 0.71 | 0.72 | 0.014 |
| **CU-ESSR-22** | **4S41188938** | **F:ACCAAGAACCACAACAAATC R:CCTAAAACCACAATCTGAGAA** | 5 | 3.70 | 0.74 | 0.79 | 0.063 |
| **CU-ESSR-23** | **4S41189019** | **F:GGGCTAATGTATCAGAGCTAA R:GCTCTAAATTGGTGATTTTCA** | 4 | 3.12 | 0.66 | 0.73 | 0.096 |
| **CU-ESSR-24** | **4S41191526** | **F:GATTGGCAGATACCTCATACA R:CAGGTTCAATCAAACATCAAT** | 5 | 4.20 | 0.75 | 0.80 | 0.063 |
| **CU-ESSR-25** | **4S45378466** | **F:AGACAGACATCCTCCTTTCTC R:GAGACAGAGAAGCAAGTTGAA** | 3 | 2.83 | 0.69 | 0.75 | 0.080 |
| **CU-ESSR-26** | **4S45378492** | **F:CTCTCTGACCTTTTCTTTTCC R:AGATAGGCTGTTGTTCCTTTC** | 4 | 3.03 | 0.82 | 0.86 | 0.047 |
| **CU-ESSR-27** | **4S47028353** | **F:CATTCAGTTCTTCTCTTCTGC R:GGAGAGATCGAGAACCAGTAT** | 4 | 3.12 | 0.800 | 0.847 | **0.055** |
| **CU-ESSR-28** | **4S49713883** | **F:TTCGTCATTTCTATCATTTCC R:AGGACTTCCATTGTTCATCTT** | 6 | 4.03 | 0.800 | 0.858 | **0.068** |
| **CU-ESSR-29** | **4S60429503** | **F:ACCTCGAGTCTTTCACTCTTC R:GAGGACCGTTGTAGTATCCTT** | 5 | 3.57 | 0.740 | 0.779 | **0.050** |
| **CU-ESSR-30** | **4S60767943** | **F:ATCTTCGCTCCTTCTCTGTT R:GGTGATGAGAGCTGAGTAGTG** | 3 | 4.76 | 0.340 | 0.437 | **0.222** |
| **CU-ESSR-31** | **4S60767994** | **F:GATGATGACGATGAAGAAGAG R:GGAGCTAAAGGATTGTCATCT** | 4 | 2.70 | 0.617 | 0.654 | 0.057 |
| **CU-ESSR-32** | **4S60768063** | **F:AATTACCCACAAAAAGAATCC R:ACTTCCTCATTCCTGGTAAAT** | 3 | 3.76 | 0.460 | 0.484 | 0.050 |
| **CU-ESSR-33** | **4S60768111** | **F:TAATTCGCAAGGATTAAGAGA R:GGTCCATGTGATATTCGTGTA** | 6 | 4.57 | 0.700 | 0.734 | 0.046 |
| **CU-ESSR-34** | **4S63721981** | **F: CTATGACGGGCAAGATTTAC R: AGAGAAAGGCTGAGAGAAAAA** | 3 | 4.18 | 0.52 | 0.55 | 0.055 |
| **CU-ESSR-35** | **4S63722896** | **F: TGTTATACTCAGCCAGTCACC R: TGGTTGGGTTGATATAGTAGG** | 4 | 3.71 | 0.62 | 0.67 | 0.075 |

### 3.3.1 Clustering Analysis of the Genotypes

Clustering analysis based on Neighbor-joining essentially allowed the detection of five major clusters of different size, further subdivided in other small clusters (Figure5.4.2.4.B). The first cluster (red) contained 11 genotypes in which 9 were High yield varieties of different zones of India. Remaining two genotypes were CU new developed high yielding varieties. Second cluster (green) which is the largest cluster, contained 12 high yield varieties. This cluster contained 8 CU new developed high yielding varieties and 4 exotic collections, including two from USA and one from Bulgaria and Bangladesh. Third cluster (blue) contained 9 genotypes. In this cluster high yielding varieties of different zones of India was noticed. Only one accession from USA and one local land races was noticed. Cluster four (yellow) contained 10 genotypes was present. Indigenous land races was dominate in this cluster. 0ut of 10 genotypes 8 genotypes belongs to indigenous land races collection. Cluster five (violet) was smallest cluster among them but appeared to be much diversified, with 7 genotypes, including two from Bangladesh and USA and one from Bulgaria. Some new releases from different Indian breeding program such as CUHY-36 and Savitri. In this cluster exotic collection was dominant.

The two main genetic groups identified by the Bayesian analysis corresponded to the developed varieties and land races gene pools. These two groups could also be further sub divided into small groups. Detailed study revealed that accessions from different country did produce any separate cluster. Some accessions were grouped in one cluster but reaming accessions were placed in different cluster. The clustering pattern indicated that geographical origin did not play role in cluster composition even at molecular level. Wu et al. (2014b). suggested that domestication along with advanced plant breeding techniques have likely narrowed the genetic basis of cultivated sesame. Many newly developed sesame varieties were bred with a few number of landrace in their pedigree. The genetic variation in sesame was consequently reduced by genetic drift and selection. Characterization of genetic diversity of available landraces especially the indigenous and exotic collection by molecular markers is of great value to assist parental line selection and breeding strategy design (Wu et al. 2014b). The understanding of these landraces can provide a better foundation for further conservation and utilization of these resources. These clusters, however, could also be associated with local adaptation, diversifying selection, familial relatedness, or combinations thereof (Yu et al., 2006). Many species have

undergone a long and complex period of domestication and breeding with limited gene flow, and this could be expected in the structure of this complex population (Sharbel *et al.*, 2000). Hence, despite the diversity observed in sesame, genetic bottlenecks may have occurred during sesame dissemination (Fernández i Martí et al., 2015). The presence of population stratification and unequal distribution of alleles could result in non-functional, spurious associations (Pritchard and Rosenberg, 1999). In order to understand the distribution of genetic diversity in the sesame cultivars, the model-based clustering approach was implemented in STRUCTURE to infer.

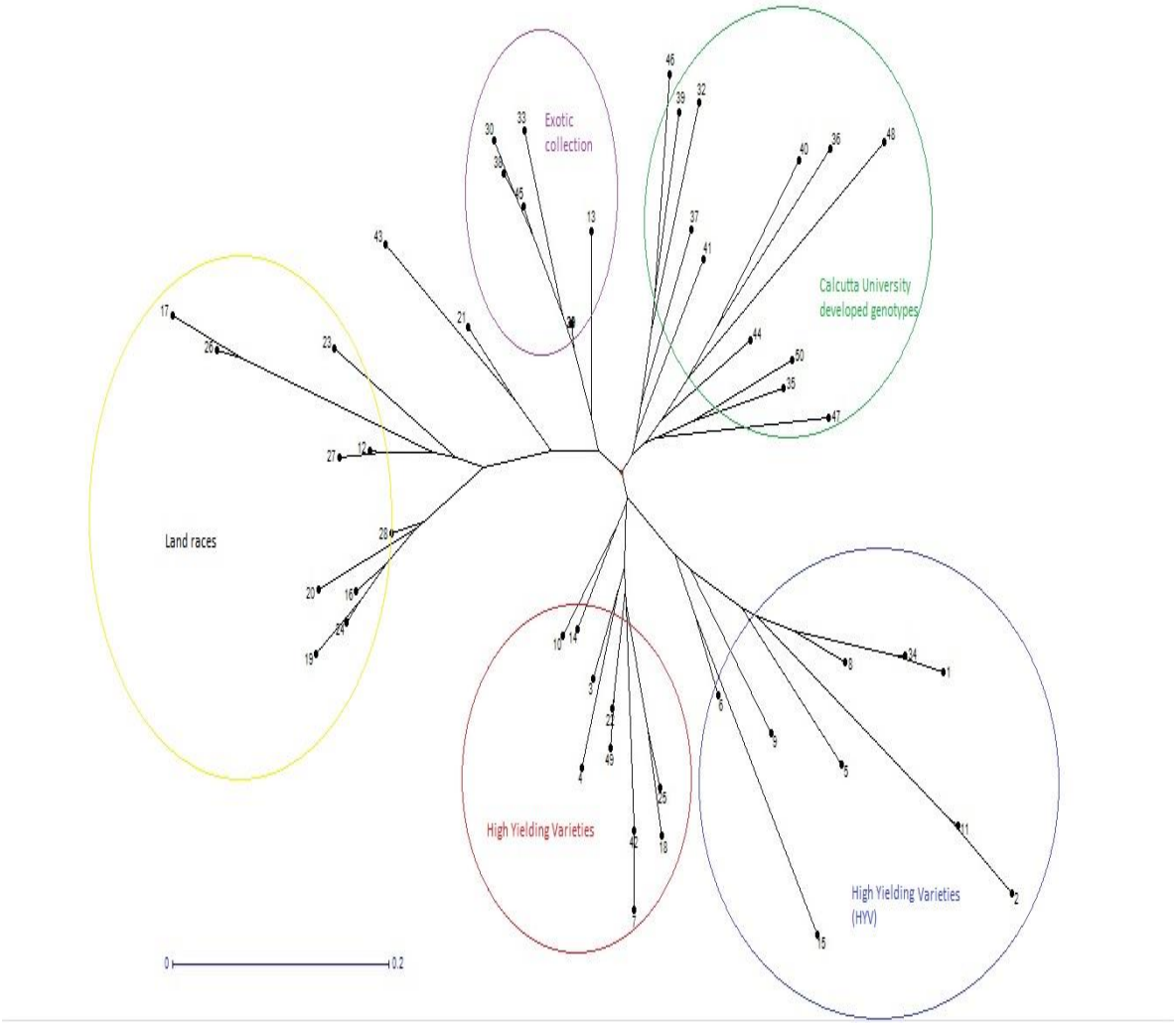



**Figure: 3.3.1.BNeighbour joining tree of 50 sesame accessions based on 35 SSR markers.**

3.4 **Population structure and LD of the marker pairs:**

The population structure was determined using STRUCTURE software, with K values ranging from 1–10. The LnP (D) value increased continuously with no obvious inflexion point before the panel was divided into 5 subgroups. However, the Δk value decreased rapidly at K = 2 and K = 3, and the locus frequency divergence among the subpopulations (Net nucleotide distance) was significant at k = 2, but not at k = 3. (Fig. 3.4.3.) shows that Δk presented a second peak for K = 5, indicating that this panel could be continuously further divided until into 5 subgroups. Pritchard et al. (Lindsey et al 2003) suggested focusing on values of K that capture most of the structure in the data and that seem biologically sensible when the model choice criterion continues to increase with increasing K. To avoid an over corrected population structure that would lead to the disappearance of the association loci in the association analysis (Nordborg,M., and Tabare,S.2002).

Approximately 9.36% of the marker pairs showed significant LD, with p values lower than 0.05 (3.4.2 Table). Approximately 18.90% of the collinear marker pairs showed significant LD, and 40.50% of the obtained LD values ($r^2$) were greater than 0.1. Approximately 8.87% of the non-collinear marker pairs showed significant LD, and 3.45% of the LD values ($r^2$) were greater than 0.1. Most of the significant LD values were higher than 0.2 were obtained from collinear marker pairs (Table 3.4.5). The LD value ($r^2$) decreased rapidly at genetic distances of less than 10 cM. The longest genetic distance between markers was 108 cM. The average genetic distance between markers was 8.58 cM and 5.76 cM for r2 > 0.1 and r2 > 0.2(Fig. 2).

### 3.4.1 Association mapping

For all the traits we applied an MLM (+ kinship + Q-matrix) model to analyze the datasets derived from the 25 collections at all locations. Only markers showing significance in more than one environment were used to further test significance through the FDR and BF min. To compare the results of the GLM and MLM, we also used a GLM (+ kinship) model to analyze the all datasets and conduct permutation testing.

Fourteen markers tolerated the FDR test in one or more environments, including 12 for oil traits. Two marker loci presented moderate-to-strong or strong-to-very strong evidence for association indifferent environments. Six markers for oil traits passed the permutation test at the 0.05 level in the GLM analysis.

### 3.4.2 Genetic diversity and population structure

To maintain relatively high levels of polymorphism and to take advantage of association mapping, different ecotypes from Calcutta University collection, mutants lines derived from various breeding programs, and some progenies of intra- and interspecies crosses were employed

in this panel. The results revealed an average genetic diversity, PIC and locus number of 0.36, 0.30 and 2.63, respectively. A low genetic diversity was found.

### 3.4.3 Linkage disequilibrium

A successful association analysis depends on knowing the precise LD status of a population. In the present study, 9.36% of the marker pairs showed significant LD values, while 18.90% of collinear and 8.90% of non-collinear marker pairs showed significant LD. Among the collinear marker pairs, 29.2% showed LD values ($r^2$) greater than 0.2.For the non-collinear marker pairs, this ratio was 0.5%. Further examination of the LD data revealed that approximately 80.5% of moderate LD ($0.2 < r2 < 0.4$) and 91.5% of strong LD ($r2 > 0.4$) was caused by linkage. Our results also showed that approximately 43.6%of moderate LD ($r^2 > 0.1$) was caused by other factors in this panel. LD resulting from non collinear marker pairs has been previously described (Fernandez et al ;2012a, Flint-Garcia et al;2003, Font I Forcada et al 2012, Pritchard et al 1999; Fernandez-Cuesta et al 2012a) provided several possible explanations for LD between non-collinear markers, including selection, co-selection of loci, population stratification, and relatedness, genetic drift or bottle necks. These elements might also generate LD values leading to spurious marker-trait associations (Sanchez-Perez et al; 2007.,Sharbel et al 2000), indicating the necessity of seriously considering population structure (Q) and relatedness (K) when conducting population-based association mapping in sesame germplasm resources (Fernandez-Cuesta et al 2012a).

In the present study, the observed LD value ($r^2$) rapidly decreased when the genetic distance was less than 10 cM. The speed of population LD decay was 8.58 or 5.76 cM for $r2 > 0.1$ or 0.2, respectively. The LD decay block was similar to that described in recent association analysis studies (Flint-Garcia et al; 2003,.Font i Forcada et al 2015,.Font i Forcada et al 2012). We selected markers that were spaced approximately 10 cM apart from the frame linkage map (Fonti Forcada et al 2013,.Ganopolus et al;2012). Because of the shortage of polymorphism markers, there were some gaps of more than 15 to 46.8 cM along the 26 chromosomes. Although more markers are needed to conduct genome-wide association analyses (GWAS) of complex traits, the size of the LD blocks would guarantee that the identified SSR markers would be sufficient for MAS in sesame breeding programs because increasing the number of markers per chromosome does not necessarily result in a stronger response to selection, particularly at a shorter distance between markers.

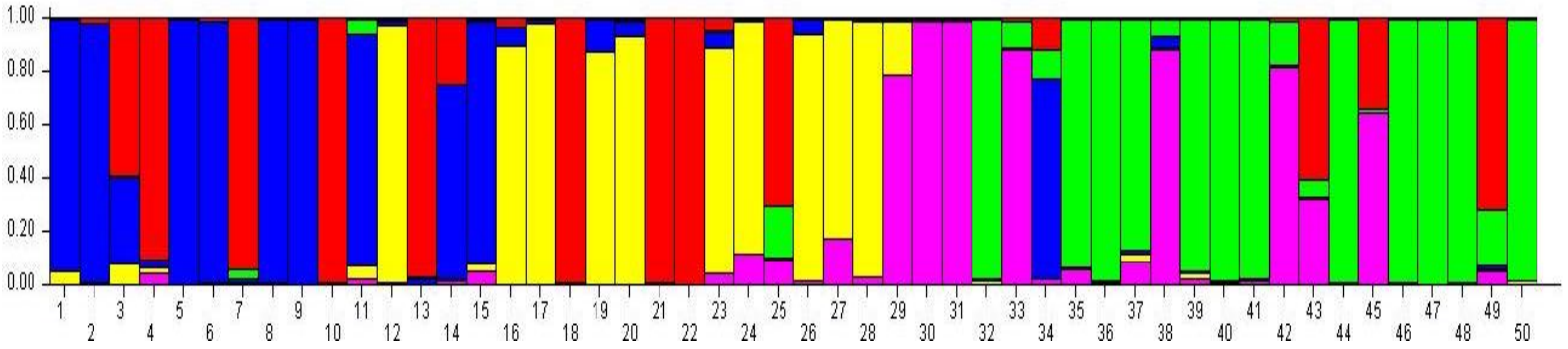



**Figure 3.4.1 Estimated population structure of sesame genotypes according to STRUCTURE software. Each individual is represented by a thin vertical segment, which can be partitioned into 4 gray-scale colored segments that represent the individual estimated membership to the 5 clusters**

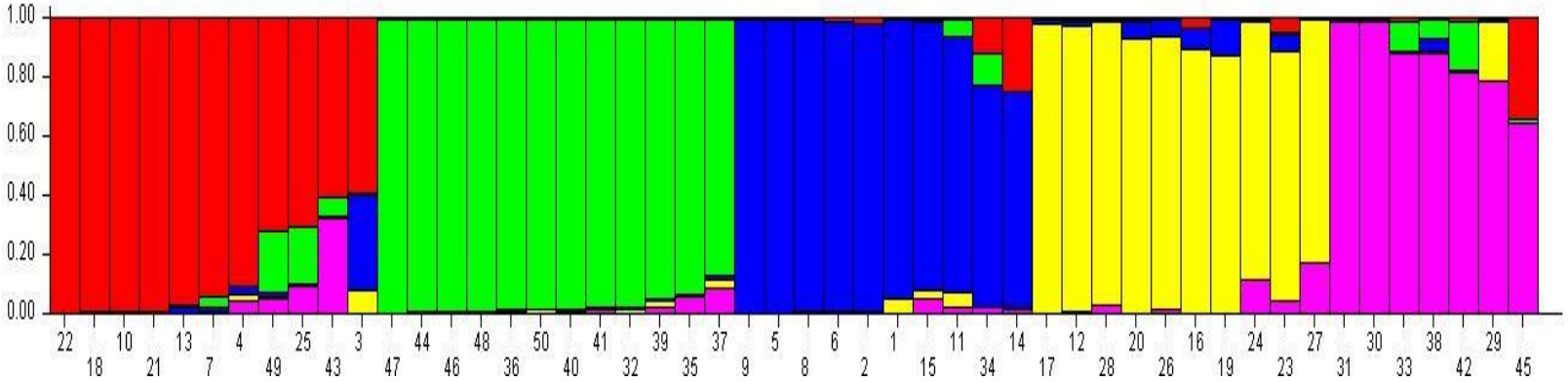



**Figure 3.4.2 Population structure of 50 sesame genotypes : Red, Green, Blue , Yellow and Violet bars corresponds to membership fraction of subpopulation 1, 2 ,3 ,4 and 5.**

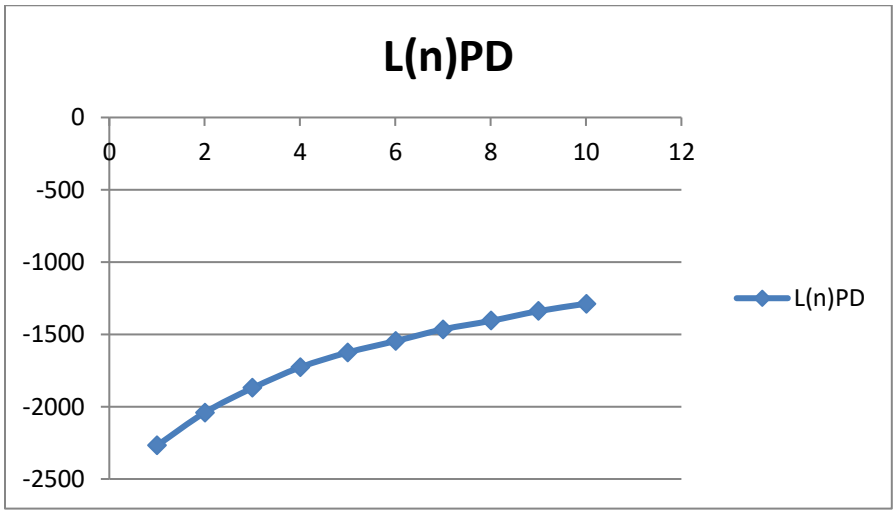



Fig 3.4.3. Estimated LnP(D) and ΔK from 10 iterations obtained through STRUCTURE analysis. (a) LnP(D) for k values from 1 to 10 for simulations using all 50 collections. (b) ΔK for k values from 2 to 9 for all 50 collections

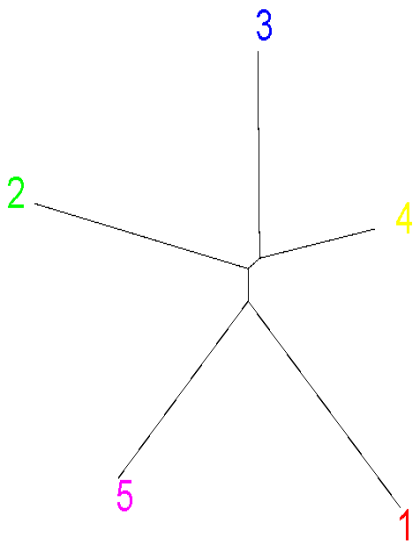
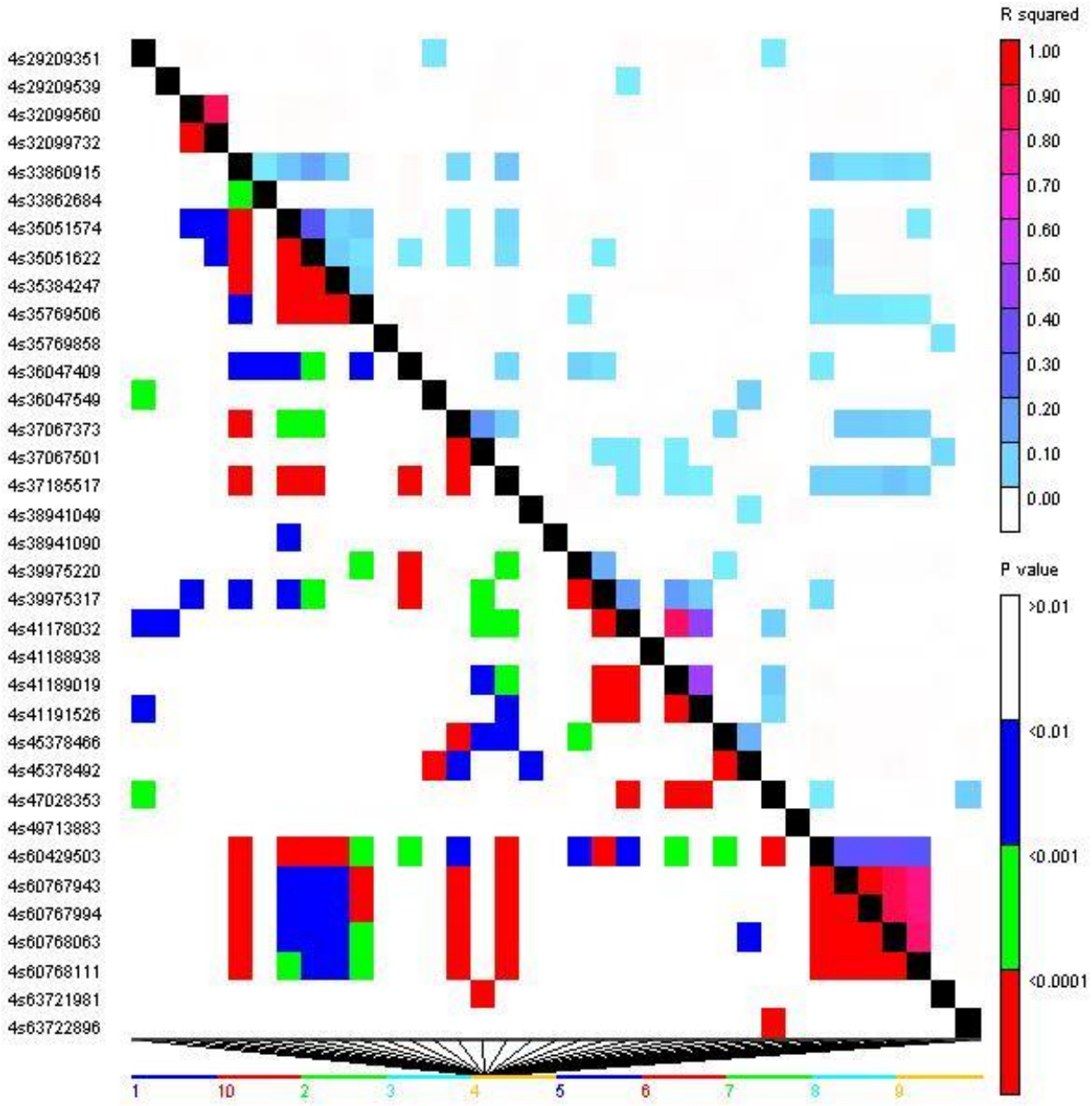



**Fig 3.4.4 Neighbor joining tree of 50 sesame accessions based on 35 SSR markers.**




3.4.5 **LD plot based on 35 SSR screened on 50 sesame accessions.**

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Dgupta 22/3/2018

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**REGISTRAR** 26/3

**(Seal)**

Registrar
University of Calcutta

# University of Calcutta

## Senate House, Kolkata - 700073

Registration Number : **8482/Ph.D.(Ag.)Proceed/2014**

Date of Registration : **17th December 2014** Date of Letter : **19th December 2014**

(Please quote the above Number and Date in all future Correspondence)

From:

The Registrar,
University of Calcutta

To:

**Sri Adil Iqbal**
**31G, Topsia 2nd Lane,**
**Kolkata-700039.**

Dear Sir,

I am desired to inform you that you have been granted registration for the Ph.D. programme under this University in

**Genetics and Plant Breeding**

in terms of 4.8 of the Regulations for the Degree of Doctor of Philosophy (Ph.D.).

This registration shall remain valid for next five years with effect from the date of registration as indicated above.

You are to comply with the usual rules of migration in case you have passed the qualifying examinations for the Ph.D. programme from a University/Institute other than the University of Calcutta.

Title of Thesis

Development Of Microsatellite Markers And Study Of Genetic Divergence In Sesame ( *Sesamum indicum* L.)

Name of the Supervisor : **Prof. Tapash Dasgupta**

Name of the Joint Supervisor : **X**

Name of the Associate Supervisor : **X**

Yours faithfully,

Registrar

23/12/2014

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# Genetic Estimates of Morphological Traits and Phenotypic Diversity in Core Collections of Sesame (*Sesamum indicum* L.)

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## Abstract

Diversity in the sesame core collection (158 genotypes), representing all eco-geographical regions, for a range of number of morphological characters was studied. Wide variation in plant habit (plant height and branching pattern), number of capsules per plant, number of seeds per capsule, mean seed weight, and yield per plant was recorded. The estimates of genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) revealed that GCV was less than its corresponding estimates of PCV for seed yield and yield component exhibiting consistent environmental interaction for all traits. Heritability estimate in broad sense revealed that seed yield per plant, 1000 seeds weight, branches per plant, capsule per plant were characterized by high estimates, while plant height, days to flowering, days to maturity and capsule length were moderately heritable. Interestingly, seed yield per plant, branches per plant, and capsule per plant exhibited both high GCV and heritability indicating that additive genetic effect was more prevalent in these traits. Morphological multivariate analysis based on 8 traits, exhibited that 158 genotypes could be grouped into two major clusters. The genetic divergence values for each pair of genotypes based on morphological data was analyzed. Highest GD was recorded between S –O434 & EC-335004 followed by EC 335004 & NIC 10645-1, IC 204063 & EC335004 and while lowest GD was observed between IC 43033 & EC 204704(44) followed by Tillotama & B14, DSS 9 & Tillotama. Selection of parents based on parents belonging to different cluster group is more preferred to get better heterotic expression Selection of parents for hybridization based on genetic diversity would likely generate desirable segregants in segregating generation.

## Introduction

Sesame (*Sesamumindicum* L.; Family: Pedaliaceae) is one of the most ancient oilseed crops known and used by mankind. It was cultivated and domesticated on the Indian subcontinent since Harappan and Anatolian eras over 4,000 yrs ago. (Bedigian and Van der Mesen, 2003).Due to the great stability of its healthy oil, easiness of extraction and resistance to drought Sesame was popular in the ancient world. Sesame ranks fifth for important edible oil crop in India after groundnut, rapeseed-mustard, sunflower and soybean. (www.agricoop.nic.in). India is the largest producer of sesame in the world. India occupies top position in the world in sesame-acreage (24%) and contribution in export (40%) (FAO Statistics Division, 2010; Raikwar and Srivastva, (2013). Interestingly, among all the Indian states West Bengal is most favoured for sesame production and productivity (Annual report, Directorate of Agriculture, Govt. of India, 2009).

India, though produces maximum sesame among the world, but demand of oil seed crop in india is far below the total oil seed production. More sesame production can narrow down the demand supply gap in oil seed sector. Horizontal expansion of any crop depends congenial climate of production, soil factor and remunerative price of the crop, so that the farmers can grow it more. One of the simplest approach to improve production is to boost up productivity of the crop. Sesame productivity in India is low compared to other sesame producing countries. Genetic up gradation of any crop primarily depends on utilization of existing genetic resource. A large germplasm resource is always favoured in plant breeding program as many desirable

traits may obviously remain in the population for restructuring favourable genotypes. Frankeland and Brown (1984), advocated the concept of core collection to represent diversity. The basic idea was to constitute novel variation from large gene pool and concept has been successfully established by Ellis *et al* (1998), Malvar *et al* (2004) and Zhang *et al* (2011). Methodology for the choice of individual accessions is yet under investigation Zhang *et al* (2011). Phenotypic and genotypic coefficient of variation (PCV and GCV), broad sense heritability and genetic gain on which the breeding methods are formulated for its further improvement. Assessing germplasm for diversity has an important implication in plant breeding program. Different quantitative traits are usually pooled up together in multivariate analysis to reach towards a conclusive outcome of diversity based multivariate analysis is often used in selection of parents for hybridization program in different crops like blackgram, (Dasgupta and Das, 1984,1991), horsegram (Dasgupta, 2005), mustard (Pandey *et al* 1984)and sesame (Akbar *et al* 2011). the present study has been conducted to asses the diversity of a core collection in sesame following morphological traits.

## Materials and Methods

The 158 sesame genotypes were collected from diverse eco-geographical regions.

158 genotypes were cultivated in Kharif-13 season following Randomized Block Design (RBD) with 3 replications having 10cm spacing between plants and 40cm between rows.

## Cultural Practices

**Experimental site:** The experiment was carried out at Agricultural Experimental Farm, University of Calcutta, Baruipur during Rabi-summer season, 2013. The farm is situated at an elevation of 10 meter above sea level, at approximately $22^{0}51'$ N latitude and $88^{0}$ 24′ E longitude. The genotypes were sown on 3rd March, 2014. The physico-chemical properties of the soil were presented in Table 2 and were estimated following the methods as suggested by Jackson, (1967). The fertilizers N: P: K were applied at the rate 50:25:25 as based, during soil preparation. Manganese sulphate at the rate of 5Kg per hectare was also given as basal. Normal culture practices was followed during cultivation. Two manual weeding were done before flowering. Three irrigations were applied when ever the soil become very dry.

## Results and Discussions

Analysis of Variance (ANOVA) revealed high and significant variation for all characters under the study indicating considerable variation among the genotypes (Table 3). The estimates of genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) revealed that GCV was less than its corresponding estimates of PCV for seed yield and its related traits. PCV was estimated to be high for seed yield per plant (104.502) followed by 1000 seed weight (55.587), capsule per plant (51.918), branches/plant (40.736) and plant height (20.590), while comparatively low variability was found for capsule length, days to flowering and days to maturity (5.624). Estimates of GCV tough less than GCV showed a similar trend for the above mentioned traits. There is scope of selection for the characters. Similar finding was reported by Patil and Sherif (1966) and Reddy *et al.*, (2001). On the contrary, Moderate to low PCV and GCV values estimated for plant height, days to 50% flowering, days to maturity and capsule length corroborated with the findings of Chandrasekhar and Reddy (1993). Broad sense heritability is a very effective parameter to access the genotypic and phenotypic variance and it may have a predictive role in selection procedure (Allard, 1960). Traits with high heritability estimates can be utilized for genetic improvement as they have potential for large genetic determination (Vasline *et al* 2000).The heritability estimates were found to be high for seed yield/plant, plant height, days to maturity, 1000 seed weight, days to flowering, capsule per plant and low heritability estimate was recorded in branch per plant, capsule length (Table 4).Similar results have been reported by Parameshwarappa *et al.* (2010) in sesame for branch per plant, days to maturity, capsule length and capsule per plant. The estimates of heritability (broad sense) include both additive and non-additive gene effect and

TABLE 1. List of genotypes

| Sr No. | Name | Seed coat colour | Sr No. | Name | Seed coat colour |
|---|---|---|---|---|---|
| 1 | S-0434 | BLACK | 28 | IS – 615 | LIGHT BROWN |
| 2 | SI-1926 | BLACK | 29 | BM – 59 | BROWN |
| 3 | NIC -13598 | BEIGE | 30 | IS – 729 | BEIGE |
| 4 | MT - 67 – 61 | BLACK | 31 | S - 0022 – A | BLACK |
| 5 | GRT – 83124 | BLACK | 32 | NIC - 16328 - A | BLACK |
| 6 | ES - 71 – A | BEIGE | 33 | IS - 288 – A | BLACK |
| 7 | GRT - 8618 - 1 | BLACK | 34 | IC -56149 | BLACK |
| 8 | IC – 206661 | DARK BROWN | 35 | IC - 750 - 1 - 84 | BLACK |
| 9 | IS - 667 - 1 - 84 | BLACK | 36 | NIC – 8263 | BLACK |
| 10 | S – 0336 | BLACK | 37 | NIC - 10645 - 1 | WHITE |
| 11 | KMR – 48 | BLACK | 38 | NIC – 7899 | OFFWHITE |
| 12 | GSM – 21 | BLACK | 39 | NIC - 8554 | WHITE |
| 13 | GRT - 8630 - C | BLACK | 40 | IC - 14121 – B | WHITE |
| 14 | IS - 8480 – B | BLACK | 41 | NIC – 8399 | OFFWHITE |
| 15 | ES – 48 | BEIGE | 42 | SI – 56 | OFFWHITE |
| 16 | NIC -8202 | BEIGE | 43 | NIC – 8222 | OFFWHITE |
| 17 | NAC/25/111/42/S/1 | BEIGE | 44 | ES - 58 -1 | WHITE |
| 18 | IC – 204500 | BEIGE | 45 | SI- 72 – A | OFFWHITE |
| 19 | MT - 67 – 18 | BEIGE | 46 | SI – 1036 | OFFWHITE |
| 20 | IC – 204533 | BEIGE | 47 | SI – 1052 | OFFWHITE |
| 21 | S – 0205 | BEIGE | 48 | SI – 260 | WHITE |
| 22 | S – 0121 | BEIGE | 49 | NIC - 8399 – 1 | WHITE |
| 23 | S - 0103 – B | BEIGE | 50 | KMS - 04 - 262 | OFFWHITE |
| 24 | CAJ - SEL - 38 | BEIGE | 51 | KMS - 5 – 351 | OFFWHITE |
| 25 | S - 8197 – B | BEIGE | 52 | IS - 347 – 1 | BEIGE |
| 26 | NIC - 16227 - B | BEIGE | 53 | KMS - 5 – 371 | OFFWHITE |
| 27 | NIC - 8327 – B | BLACK | 54 | IS - 78 -1 – 1 | WHITE |
| 55 | SI - 2323 - 3 | LIGHT BROWN | 85 | V – 12 | DEEP BROWN |
| 56 | RJS – 8331 | WHITE | 86 | V – 10 | DEEP BROWN |
| 57 | KMS - 4 – 254 | OFFWHITE | 87 | SAHEB | LIGHT BROWN |
| 58 | KMR - 61 -1 | WHITE | 88 | B – 14 | BROWN |
| 59 | IS 101 | WHITE | 89 | V – 1 | BROWN |

| Sr No. | Name | Seed coat colour | Sr No. | Name | Seed coat colour |
|---|---|---|---|---|---|
| 60 | SI – 205 | WHITE | 90 | V – 15 | BROWN |
| 61 | SI - 890 – 1 | OFFWHITE | 91 | B – 76 | BROWN |
| 62 | SI – 205 | OFFWHITE | 92 | NIC – 8316 | BEIGE |
| 63 | KMR – 11 | OFFWHITE | 93 | RAMA | DARK BROWN |
| 64 | NIC – 8214 | WHITE | 94 | TILLOTAMA | DARK BROWN |
| 65 | NIC - 7905 | WHITE | 95 | SAVITRI | DARK BROWN |
| 66 | NIC – 16204 | OFFWHITE | 96 | TMV – 6 | LIGHT BROWN |
| 67 | IC – 43014 | MEDIUM BROWN | 97 | TMV – 4 | DARK BROWN |
| 68 | IS – 152 | MEDIUM BROWN | 98 | UMA | LIGHT BROWN |
| 69 | RJS – 190 | OFFWHITE | 99 | NIRMALA | BEIGE |
| 70 | IS - 187 | OFFWHITE | 100 | VRI - 1 | DARK BROWN |
| 71 | RJS - 148 - 1 - 80 | OFFWHITE | 101 | TKG - 22 | WHITE |
| 72 | KMS - 5 – 384 | BLACK +WHITE | 102 | DSS - 9 | WHITE |
| 73 | KMS - 4 – 299 | WHITE | 103 | G T - 2 | WHITE |
| 74 | KM – 10 | WHITE | 104 | GT – BLACK | BLACK |
| 75 | IC – 43110 | WHITE | 105 | AMRIT | LIGHT BROWN |
| 76 | KMS - 5 – 587 | WHITE | 106 | TKG - 352 | WHITE |
| 77 | NIC - 10621 - B | BEIGE | 107 | RT - 348 | WHITE |
| 78 | IC - 41923 – B | WHITE | 108 | IC – 131490 | MEDIUM BROWN |
| 79 | IC – 14136 | WHITE | 109 | IC – 14331 | MEDIUM BROWN |
| 80 | IC – 205312 | OFFWHITE | 110 | IC – 14053 | WHITE |
| 81 | S - 0160 – A | OFFWHITE | 111 | IC – 43033 | DARK BROWN |
| 82 | N - 60 – 276 | WHITE | 112 | IC – 204159 | WHITE |
| 83 | IC - 204843 | OFFWHITE | 113 | IC – 152485 | BROWN |
| 84 | KMR – 23 | WHITE | 114 | IC – 20477 | MEDIUM BROWN |
| 115 | IC – 17477 | WHITE | 146 | CR -11 | BEIGE |
| 116 | IC – 2621694 | MEDIUM BROWN | 147 | Black til | BLACK |
| 117 | IC – 96230 | BRIGHT BLACK | 148 | Red brown til | DARK BROWN |
| 118 | IC – 204063 | WHITE | 149 | Winter season til | DARK BROWN |
| 119 | EC – 310421 | BLACK | 150 | Orissa local | BEIGE |
| 120 | EC – 310448 | BLACK | 151 | Double skin | BROWN |
| 121 | EC – 138835 | BROWN | 152 | Red brown til | BROWN |
| 123 | EC – 100043 – A | DARK BROWN | 153 | Gujarat white til | WHITE |

| Sr No. | Name | Seed coat colour | Sr No. | Name | Seed coat colour |
|---|---|---|---|---|---|
| 124 | EC – 303442 | BROWN | 154 | Gujarat white til | WHITE |
| 125 | EC – 334988-B | BLACK | 155 | Gujarat – z-black | BLACK |
| 126 | EC – 335004 | BEIGE | 156 | Tripura small | WHITE |
| 127 | EC -334973 | BROWN | 157 | Double skin white | BEIGE |
| 128 | EC – 334971 | BROWN | 158 | Brown til | BROWN |
| 129 | EC – 335004 | DARK BROWN | | | |
| 130 | EC – 334991 | BROWN | | | |
| 131 | EC – 182832 | BLACK | | | |
| 132 | EC – 204704 | DARK BROWN | | | |
| 133 | EC – 334962 | BLACK | | | |
| 134 | EC – 303435 | DARK BROWN | | | |
| 135 | EC – 303433 | BLACK | | | |
| 136 | EC – 164966 | BROWN | | | |
| 137 | EC – 41923 – B | LIGHT BROWN | | | |
| 138 | CUHY – 57 | DEEP BROWN | | | |
| 139 | CUHY – 23 | BEIGE | | | |
| 140 | CUHY – 24 | BEIGE | | | |
| 141 | CUHY – 47 | WHITE | | | |
| 142 | CUHY - 13 | MEDIUM BROWN | | | |
| 143 | CUMS - 9 | BEIGE | | | |
| 144 | CUMS – 17 | BEIGE | | | |
| 145 | CUMS – 11 | BEIGE | | | |

TABLE 2. Physico-chemical characteristics of the experimental soil

| Mechanical analysis | Physical characteristics | Chemical analysis |
|---|---|---|
| Sand - 25% | Soil Colour- Light Brown | pH - 6.70 |
| Silt - 35% | Apparent density— 1.24gm c.c. | EC - 0.151ds/ms |
| Clay - 40% | Absolute specific gravity-2.56 | Organic carbon - 1.02% |

TABLE 3. ANOVA for Eight characters of sesame

| Source | DF | Plant height | Days to flowering | Days to maturity | Capsules length | Capsules/ plant | Branches/ plant | 1000 seed weight | Seed yield / plant |
|---|---|---|---|---|---|---|---|---|---|
| Replications | 1 | 376.000** | 94.000** | 94.000** | 0.602** | 376.000** | 60.160** | 0.338** | 330.169** |
| Treatments | 158 | 791.589** | 20.321** | 48.843** | 0.249** | 2671.405** | 8.777** | 6.139** | 91.051** |
| Error | 158 | 50.31 | 1.06 | 11.20 | 0.001 | 217.77 | 0.18 | 0.98 | 11.64 |

** significant at 1% level

TABLE 4. Mean and estimates of genetic parameters

| Estimates | plant height | days to flowering | days to maturity | capsules length | capsules / plant | branches / plant | 1000 seed weight | seed yield / plant |
|---|---|---|---|---|---|---|---|---|
| GCV % | 19.54 | 9.111 | 5.674 | 14.524 | 50.868 | 39.686 | 54.537 | 103.452 |
| PCV% | 20.590 | 10.161 | 6.624 | 15.574 | 51.918 | 40.736 | 55.587 | 104.502 |
| H% | 99 | 97.6 | 99 | 79 | 96.5 | 89 | 98.5 | 99.5 |
| MEAN | 97.16 | 34.59 | 88.61 | 2.32 | 67.16 | 5.28 | 3.21 | 8.10 |
| RANGE | 71-156.3 | 27-44 | 79-98 | 1.4-3.3 | 22-220 | 1-10 | 1.9-4.4 | 2.2-34.17 |

its higher estimates in broad sense indicates that the trait is least influenced by environmental effects (Shim, *et al.*, 2001).

High GCV coupled with high heritability can provide more desirable information than a single parameter alone (Saha *et al.*, 1990) and would like to indicate that the traits were controlled by additive genetic effects (pham *et al* 2011) Hence in this study seed yield / plant, 1000 seed weight, branch/ plant and capsule/plantin the present study showed high GCV and heritability as well. This highlighted that for all these four traits additive genetic effects were more important.

All 158 genotypes were further analysed by NTSYS version 2.20 for grouping. Two distinct clusters were found. (Figure 1 and Table 5). Cluster I comprised 157 genotype where as cluster II consisted of only one genotypes. Two distinct sub-clusters were identified for cluster I. The sub- clusters IA and IB comprised containing 1 and 156 genotypes respectively. Cluster IB has been further grouped into two distinct sub cluster IB-I and IB-II comprising 154 genotypes and 2 genotype respectively. The genetic dissimilarity values for each pair of genotypes based on molecular data showed maximum divergence between S – o434 and EC-335004 followed by EC 335004 and NIC 10645-1, IC 204063 and EC335004 and while lowest GD was observed between IC 43033 & EC 204704 followed by Tillotama & B14, DSS 9 & Tillotama. desirable segregates are expected if crossing is done between genotypes with high dissimilarities coefficient. Thus hybridization program between S – o434 and EC-335004 followed by EC 335004 and NIC 10645-1, IC 204063 and EC335004 would leads to promising breeding materials which may lead to high yielding varieties.

TABLE 5. Cluster composition of 158 genotypes

| CLUSTER | SUB CLUSTER | NUMBERS OF GENOTYPES | ACCESSION NUMBER |
|---|---|---|---|
| CLUSTER-I | A | 1 | 1 |
| | B I | 153 | 2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22, 23,24,25, 26,27,28,29,30,31,32,33,34,35, 36,38,39, 40, 41 ,45,46,47,48,49,50, 51,52,53,54,55,56, 57,58 ,59, 60, 61, 62,63,64,65,66,68,69,70,71 72,73, 74,75, 76, 78,79, 80,81,82,83,84,85,86,87, 89,90,91,92,93, 94, 95,96, 97,98, 99,100,101,102, 103,104, 105,106, 108,109, 110, 111, 112, 113,114,115,116,117,118,119,120,121,122,123,124,125,126, 127,128,129, 130,131,132, 134,135,136, 137, 138,139,140,141, 142, 143,144,145,146, 147, 148,149,150,151, 152, 153.154, 155, 156, 157, 158 |
| | II | 2 | 37, 67 |
| CLUSTER-II | | 1 | 107 |

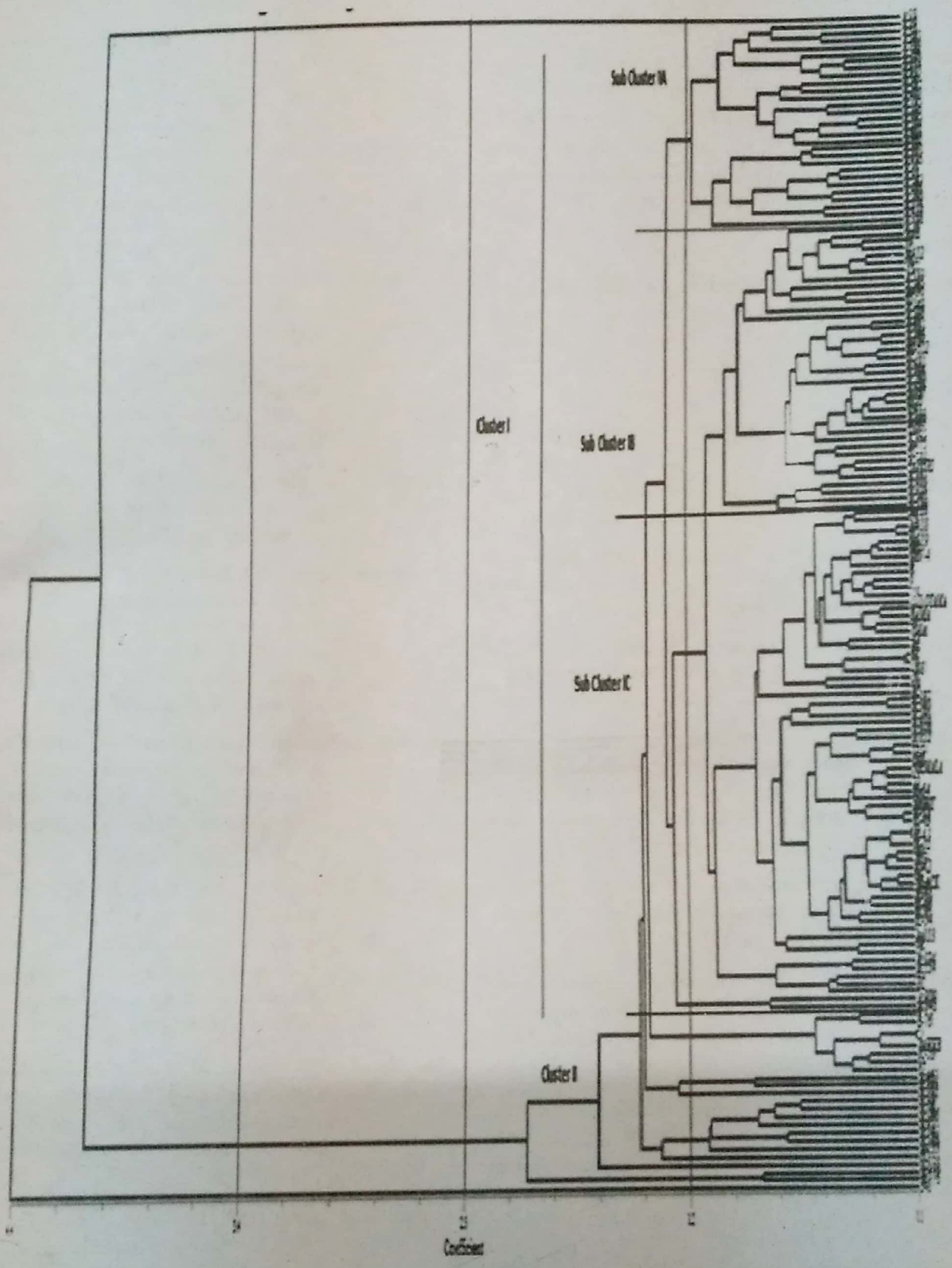


Fig. 1 Dendrogram of 158 core collection in sesame

An investigation of the cluster composition revealed that each of the two clusters consisted of varieties belonging to different origin, i.e. from different states of India and also from different countries (Table 5).This indicate that genetic divergence of genotypes is independent of geographic origin. Similar finding was also reported Banerjee and Kole (2009b).

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# Genetic estimates and diversity study in Sesame(*Sesamum indicum L.)*

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***Abstract:*** *Genetic estimates and diversity of 33 genotypes sesame representing different eco-geographical regions were studied for number of morphological characters. Wide variation in plant habit (plant height and branching pattern), number of capsules per plant, number of seeds per capsule, mean seed weight, and yield per plant was recorded. The estimates of genotypic coefficient of variation (GCV) were less than its corresponding estimates of PCV for seed yield and yield component, exhibiting consistency in environmental interaction for character expression. Capsule per plant exhibited high heritability coupled with good genetic advance indicating that additive genetic effect was more prevalent in this trait. Correlation study at phenotypic level showed that seed yield per plant was significantly and positive associated with plant height, number of branches per plant, number of capsule per plant and capsule length. So selection for high no of capsule per plant, capsule length and no of branches per plant will lead towards high yield. The genotypes were further grouped through multivariate analysis based on 8 traits. The genotypes constituted three major clusters. Origin of genotypes did not play a significant role in constitution of clusters. Selection of parents based on parents belonging to different cluster would like to produce more desirable segregants.*

***Keywords:*** *Cluster, Correlation, Genetic variability, Heritability and Sesame*

## I. Introduction

Sesame is one of the most ancient and important oilseed crops grown from ancient times. It was cultivated and domesticated on the Indian subcontinent during Harappan and Anatolian eras over 4,000 yrs ago. (Bedigian and Van der Mesen, 2003). Due to the stability of its healthy oil, easiness of extraction and resistance to drought, sesame was popular in the ancient world, Sesame is considered as a nutritious oilseed crop being rich source of protein (18–25 %), carbohydrate (13.5 %), minerals and polyunsaturated fatty acid (Bedigian et al. 1986). Sesame oil is favoured as a media of cooking by Indians and Africans. Presence of sesamol, a unique anti-oxidant and more poly-unsaturated fatty acid such as oleic acid (43 %), linoleic acid (35%), palmitic acid (11%) and stearic acid (7%), have made it to 'queen of oilseed crop' (Ashri 1989; Fukuda et al. 1986). Sesame ranks fifth for important edible oil crop in India after groundnut, rapeseed-mustard, sunflower and soybean. (www.agricoop.nic.in). India holds top position in the world in sesame-acreage (24%) and contribution in export (40%) (FAO Statistics Division, 2012) Raikwar and Srivastva, (2013). Productivity of sesame in India is low compared to other sesame producing countries. Improvement in productivity will definitely boost the oil crop market of India and other ancillary industries. One of the simple approach to improve production of any crop is to boost up productivity, Genetic up gradation of any crop depends primarily on utilization of existing genetic resource. A large germplasm resource is always favoured in plant breeding program as many desirable traits may obviously remain in the population which may exploit breeding program. Study on genetic diversity comparatively limited in sesame. Several genetic parameters, such as, phenotypic and genotypic coefficient of variation (PCV and GCV), heritability and genetic gain help to assess genetic diversity of experimental materials.

Multivariate analysis is an important biometric technique when different quantitative traits are usually pooled up together to reach towards a conclusive outcome of diversity. Multivariate analysis is often used in selection of parents for hybridization program in different crops like blackgram, (Dasgupta and Das, 1984,1991), horsegram ( Dasgupta et al 2005), mustard (Pandey et al 1984) and sesame (Tripathi et al 2013, Akbar et al 2011). The present study has been conducted to assess the diversity genotypes in sesame following different genetic estimate and multivariate analysis.

## II. Material & Method

33 sesame genotypes were collected from diverse eco-geographical regions of India and abroad. The genotypes were grown in summer season following Randomized Block Design (RBD) with 3 replications having 10cm spacing between plants and 40cm between rows at Agricultural Experimental Farm, University of Calcutta, Baruipur in 2015. The farm is situated at an elevation of 10 meter above sea level, at approximately $22^0 51^/$ N latitude and $88^0 24^/$ E longitude. The N: P: K fertilizers were applied at the rate 50:25:25 as basal dose

during final soil preparation. Manganese sulphate at the rate of 5Kg per hectare was also given as basal. Normal culture practices were followed during cultivation and irrigations were applied whenever the soil becomes very dry. Statistical cluster analysis of 33 genotypes with respect to morphological characters was done by Average Linkage Between Groups (in other words UPGMA) Method. The divergence between accessions was evaluated using a Euclidean distance dissimilarity matrix. Euclidian distance between lines were calculated and UPGMA cluster analysis was performed with the help of IBM-SPSS software (Version: 16.0) for dendrogram formation

## III. Result & discussion

Eight morphological characters were studied and ANOVA (Analysis of Variance) revealed that genotypes were significantly different from each other indicating high diversity among the genotypes.

Phenotypic coefficients of variation exhibited marginal higher values but maintained a close relation with genotypic coefficients of variation for all the traits. Highest coefficients of variation (phenotypic and genotypic) were exhibited by capsule per plant, seed yield/plant ( Table 2) Sumathi and Murlidharan (2010), Parameshwarappa *et al.*, (2009) and Sudhakar *et al.* (2007), and number of primary branches/plant similar finding was reported earlier Solanki and Gupta, (2003), Sudhakar *et al.* (2007), Saha *et al.* (2012), Gidey *et al.*, (2013), Iqbal and Dasgupta, (2015), in sesame. High GCV and PCV for capsule/plant, seed yield/plant and number of primary branches/plant suggest reasonably high variability in the studied materials and this would obviously help in upgrading the genotypes by simple selection. Heritability in broad sense was highesr for 1000 seed weight followed by capsule per plant and seed yield/plant. Moderately heritability was observed for capsule length, plant height and days to 50% flowering on the contrary days to maturity exhibits very low heritability. Information on heritability of any traits aids to selection as traits with high heritability would like to exhibit similar character experrsion consistently over years. High heritability with high GCV of any trait is favourable combination for genetic up gradation of any trait as better estimates simultaneously in two biometrical parameter of any trait indicate that the genetic control of the trait is additive in nature( Pham et al 2011). The trait Capsule/plant followed by seed yield/plant showed such ideal combination in genetic estimates. High heritability with genetic advance of any trait point out that the trait is under additive genetic control. In self pollinated crop additive gene effect is more desirable than non-additive genetic effect. Capsule/plant showed high GA, high heritability and also GCV. So, capsule/plant exhibits innate potentiality for genetic improvement in sesame. Such combination was not observed in any other trait. Two other traits namely 1000 seed weight and seed yield/plant were characterized by high heritability with moderate genetic advance, moderate heritability with low genetic advance were found for plant height, branches per plant and days to flowering. The results confirm previous finding of Reddy et al (2001) , Iqbal and Dasgupta (2015).

Correlation study ( genotypic and phenotypic ) were showed that plant height, number of branches per plant, no of capsule per plant and capsule length were positively and significantly correlated with seed yield per plant ( Table 3). The results are in concurrence with the results of Uzun and Cagirgan (2001), and Sumathi et al. (2007) Subramanian and Subramanian (1990).Thus selection of any of these characters would lead to the improvement of seed yield/plant. On the contrary, 1000 seed weight showed negative relationship with seed yield parsaeian et al. (2010). .

A few inter- relationships showed significant and positive correlation namely plant height and number of branches/plant, number of capsule/plant and number of primary branches/plant, Plant height and number of capsule/plant, Capsule length and number of capsule/plant, number of capsules per plant and seed yield per plant. Negative interrelationship were found between days to 50% Flowering and 1000 seed weight, days to 50% flowering and capsule length. Days to maturity also had negative effect on capsule length and 1000 seed weight though the effect was not significant.

Combining all traits, selection for plant height would not only improve seed yield/plant but also will improve capsules/plant and branches/plant through correlated response. However in intensive cropping system the breeders are interested for early maturity though delayed maturity improves seed size.

Improvement of the trait capsule/plant would also improve plant height, branches/plant, capsule length and ultimately seed yield/plant. So, capsules/plant was the most desired trait for improvement of several traits and also seed yield/plant. It is interesting to note that capsules/plant had additive genetic control with high GCV, high GA and high heritability.

All 33 genotypes were further analyzed by SPSS for grouping. Three distinct clusters were found. (Figure 1 and Table 4). Cluster I comprised of 23 genotype where as cluster II consisted of 6 genotypes and cluster III consisted 4 genotypes. Four distinct sub-clusters were identified for cluster I. The sub- clusters IA, IB, IC and ID contained 8, 7, 2 and 6 genotypes respectively. Cluster IA has been further grouped into two distinct sub clusters IA-i and IA-ii comprising 4 genotypes and 2 genotypes each. The maximum Euclidean distance recorded between RT-346 and EC-303435(4) followed by EC-204704 and RT-346, IC-20477 and RT-346. Desirable segregates are expected if crossing is done between genotypes with high dissimilarities coefficient.

An investigation of the cluster composition revealed that each of the two clusters consisted of varieties belonging to different origin, i.e. from different states of India and also from different countries.This indicate that genetic divergence of genotypes is independent of geographic origin. Similar finding was also reported Banerjee and Kole (2009), Kandamoorthy and Govindarasu (2005), Banumathy *et al. (*2010) and Saha et al (2012).

## IV. Conclusion

Sesame genotypes showed considerable genetic variability and divergence. It was found that Additive genetic effect was more prevalent for Capsule per plant. Correlation study at phenotypic level showed that seed yield per plant was significantly and positive associated with plant height, number of branches per plant, number of capsule per plant and capsule length, so selection for high no of capsule per plant, capsule length and no of branches per plant will lead towards high yield. The cluster analysis helped in grouping the genotypes into different clusters having specific characteristic traits which may be helpful in selecting parents for future breeding programs. Crossing between the genotypes RT-346 ,EC-303435(4) , EC-204704 and IC-20477 would most likely express considerable amount of heterosis in F1 generation and also provide a wide spectrum of recombinants in segregating generations. Grouping of genotypes based on multivariate analysis was independent of origin of cultivars. The conventional assumption that selecting genotypes of different geographical origin will maximize the diversity available to a breeding project does not follow in sesame.

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**Table 1 :** List of Genotypes

| Serial No. | Name of genotypes | Origin | Serial No. | Name of genotypes | Origin |
|---|---|---|---|---|---|
| 1 | Savitri | Malda | 18 | TKG-352 | Tikamgarh |
| 2 | Rama | Malda | 19 | TKG-22 | Tikamgarh |
| 3 | Saheb | West Bengal | 20 | IC-20477 | India |
| 4 | Osc-207 | Odisha | 21 | IC-26230 | India |
| 5 | Cums-20 | West Bengal | 22 | IC-141464 | India |
| 6 | DSS-09 | Karnatak | 23 | EC-182832(26) | Bulgaria |
| 7 | B-76 | South 24 parganas | 24 | EC-334988(3) | Bulgaria |
| 8 | V-13 | West Bengal | 25 | EC-303435(4) | Bulgaria |
| 9 | V-15 | Hoogly | 26 | EC-161492-A | Bulgaria |
| 10 | CST-2001 | West Bengal | 27 | EC-164966(50) | USA |
| 11 | Haveri | West Bengal | 28 | EC-204704 | USA |
| 12 | Utawadia | West Bengal | 29 | EC-41923(B) | USA |
| 13 | Cums-11 | West Bengal | 30 | EC-303433 | USA |
| 14 | Cums-9 | West Bengal | 31 | EC-303442(32) | Bulgaria |
| 15 | VRI-1 | Tamil Nadu | 32 | EC-100043-A | Bulgaria |
| 16 | RT-346 | Rajasthan | 33 | EC-100049(3) | Bulgaria |
| 17 | GT-Black | Gujarat | | | |

**Table.2.** Estimates of variability, Heritability and Genetic Advance in sesame

| | 50% Flowering | Days to Maturity | 1000 Seed Weight | Plant Height | Branches per Plant | Capsule Length | Capsule per Plant | Seed yield Per Plant |
|---|---|---|---|---|---|---|---|---|
| **GCV** | 5.76 | 1.97 | 26.11 | 14.64 | 29.31 | 13.15 | 62.56 | 36.37 |
| **PCV** | 7.92 | 4.34 | 26.30 | 20.73 | 42.21 | 16.34 | 67.70 | 45.32 |
| **H%** | 52.88 | 20.61 | 98.57 | 49.9 | 48.21 | 64.79 | 85.39 | 64.41 |
| **GA** | 2.69 | 0.74 | 1.27 | 13.57 | 1.63 | 0.38 | 72.09 | 2.14 |
| **Mean** | 42.98 | 88.899 | 2.479(gm) | 90.111(cm) | 5.596 | 2.176(cm) | 65.576 | 4.383(gm) |
| **Range** | 36.33-48.00 | 83.00-94.67 | 1.64-3.13 | 70.33-121.67 | 2.67-8.00 | 1.57-3.07 | 29.00-193.00 | 4.04-8.22 |

**Table- 3:** Phenotypic Correlation Matrix

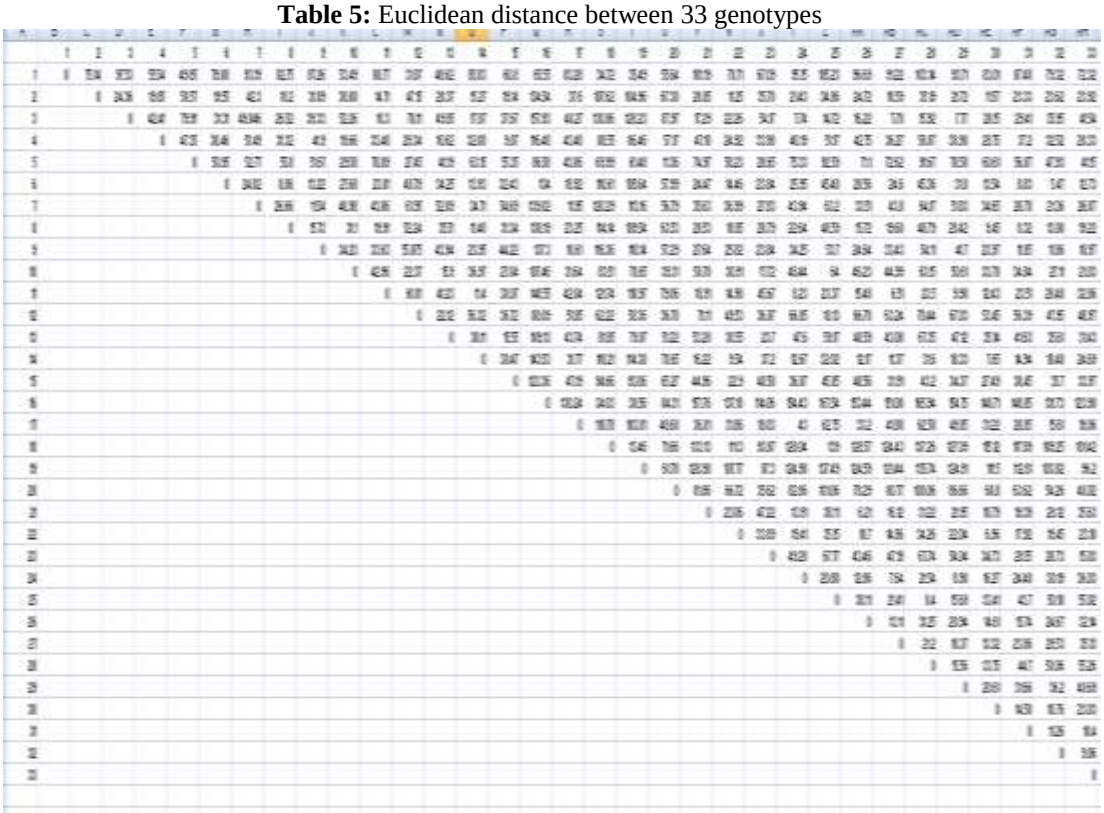
| | **50% flowering** | **Days to maturity** | **1000 Seed weight** | **Plant Height** | **Branches per Plant** | **Capsule Length** | **Capsule per Plant** | **Seed yield Per Plant** |
|---|---|---|---|---|---|---|---|---|
| **50% flowering** | 1.000 | | | | | | | |
| **Days to maturity** | 0.285 | 1.000 | | | | | | |
| **1000 Seed weight** | -0.120 | 0.049 | 1.000 | | | | | |
| **Plant Height** | 0.353* | 0.240 | -0.056 | 1.000 | | | | |
| **Branches per Plant** | 0.149 | -0.050 | 0.058 | 0.151 | 1.000 | | | |
| **Capsule Length** | 0.234 | -0.138 | -0.153 | -0.065 | 0.044 | 1.000 | | |
| **Capsule per Plant** | 0.232 | 0.052 | -0.026 | 0.080 | 0.646** | 0.088 | 1.000 | |
| **Seed yield Per Plant** | 0.613** | 0.074 | -0.070 | 0.461** | 0.435** | 0.414** | 0.536** | 1.000 |

*and ** : significant at 5% and 1% level respectively

**Table 4:** Cluster composition of 33 genotypes

| CLUSTER | SUB CLUSTER | | NUMBERS OF GENOTYPES | ACCESSION NUMBER |
|---|---|---|---|---|
| CLUSTER-I | A | i | 8 | DSS-09, EC-100043-A, V-13, EC-303442(32), IC-141464, EC-303433, Cums-9, Rama |
| | | ii | 7 | IC-26230, EC-161492-A, Haveri, EC-164966(50), Saheb, EC-334988(3), EC-41923(B), |
| | B | | 2 | EC-303435(4), EC-204704 |
| | C | | 6 | V-15, GT-Black, B-76, CST-2001, EC-182832(26), EC-100049(3) |
| | D | | 4 | Osc-207, Cums-11, VR-I, Utawadia |
| CLUSTER- II | | | 2 | Cums-20, IC-20477 |
| CLUSTER- III | | | 4 | Savitri, TKG-352, TKG-22, RT-346 |

**Table 5:** Euclidean distance between 33 genotypes



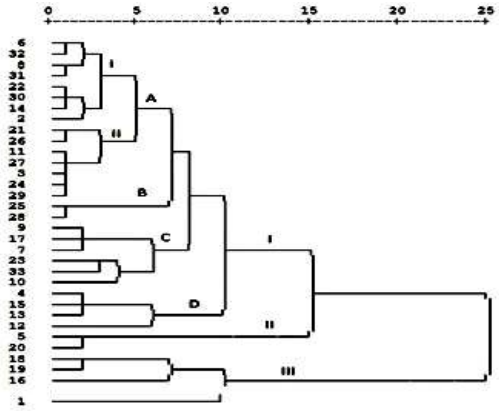



**Fig: 1** Dendrogram showing the distribution of 33 genotypes in clusters

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**RESEARCH ARTICLE**

# ASSOCIATION OF SEED IRON AND ZINC CONTENT WITH SEED YIELD AND OTHER TRAITS IN SESAME (SESAMUM INDICUM L.)

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**ABSTRACT**

Selfed progenies derived from diverse populations were evaluated in two seasons at Agricultural Experimental Farm, University of Calcutta, Baruipur, West Bengal, India to investigate the correlation of seed iron (Fe) and zinc (Zn) content with seed yield and component traits in sesame. Significant genetic variability among the progenies was observed for Fe, Zn seed yield and other component traits. Significant and positive correlation was observed between Fe and Zn. Seed yield was significantly correlated positively with plant height, capsule no/plant and 1000 seed wt. While Fe content in seed was negatively associated with capsule length. The PCA revealed that seed yield per plant had a strong relation with capsules per plant and plant height suggesting the need for more emphasis on these components for increasing the seed yield in sesame. Zinc and iron also had a strong relationship but there are no significant relationship with seed yield per plant and micronutrients.



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## INTRODUCTION

Traditional breeding for last two decades was mainly focused on breeding for high yield not only in cereals or pulses but also in oil seeds to meet the increased demand for increasing human population. In this process, nutritional value of grain or seed is often ignored. This unintentionally led to micronutrient malnutrition among poor people of developing country. World Health Organization (WHO, 2012) has figured out grain iron(Fe) and zinc(Zn) as limiting micronutrients for human health. The impact of deficiency in iron and zinc in human health is well known. Zinc deficiency induces the human health problem like impairment in physical development, brain function and immune system, weight loss, apetite loss, etc while iron deficiency leads to anemia, dizziness, headache, chest pain and frequent worm infestation. High yielding variety rich in such micronutrients may help to recovered from crop-based nutrient deficiency. But grain iron and zinc. Content are often negatively correlated with grain yield in crops like maize (Banziger and long, 2000), sorghum (Reddy *et al*., 2005) making the task of breeders arduous for developing level of micronutrient and grain yield simultaneously Sesame an important healthy oil seed crop, in several continue including India is after consumed as raw seed can mitigate malnutrition, if iron and Zn content in the seeds are enhanced.

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Clearly reassociations of micronutrients with seed yield can help plant breeders is planning breeding strategy augmenting content of Fe and Zn in seeds of sesame. So, the investigation was carried out in sesame to disclose the linkage of important nutritional components like Fe and Zn with seed yield and other quantitative traits.

## MATERIALS and METHODS

The experimental material consisted of 50 diverse genotype different parts of Indian and aboard from USA, Belgium, Bulgaria and Bangladesh grown separately in 2 experiments in RCBD with 3 replications in rabi/summer season (February – May) 2014 at Agricultural Experimental Farm, University of Calcutta, Baruipur, West Bengal India. Each genotype was grown in 4 rows with 3m long and 30 cm spacing between rows, the micronutrient levels of soil measured in six samples (Three each at 0-15 cm and 15-30 cm depth) at the time of planting varied from 6.9mg/kg Fe and 2.4mg/kg Zn for the field. Data were recorded from 5 randomly selected plants in each replication for(1) plant height (2) days to flowering (3)primary branches/plant (4)secondary branches/plant (5) Internode length (cm) (6)days to maturity (7) capsule length (cm) (8) capsule breadth(cm) (9)1000 seed weight(gm) (10)seed yield/plant. The seeds were cleaned after harvest and precautions were taken to avoid any contamination with dust particle and other extrnous matter. The seed samples were analyzed for Fe and Zn by taking the digest using Atomic

Absorption Spectrophotometer (Agilant seed Fe and Zn content, seed yield and other morphological traits were analyzed for across .two years following a fixed model ANOVA of randomized complete block design (Gomez and Gomez, 1984) using MSTAT programme. The broad sense heritability was estimated as the ratio of genotypic variance to phenotypic variance. The correlation coefficients among the micronutrients, seed yield and its component traits were estimated using standard procedure.

## RESULTS AND DISCUSSION

Average grain micronutrient across year was higher in EC – 310448(36) (231.50mg/kg Fe) and EC – 164966(50) (80.09mg/kg Zn) followed by analysis Of variance showed non-significant effect of year in Fe and Zn (Table).The difference in the soil Fe and Zn content between the two years were not reflected in the seed Fe and Zn content correlation between two season for Fe($r=0.66;p<0.01$), Zn($r=0.67;p,0.01$) was highly significant, indicating high level of Consistency in the rankings of entic across the two season for micronutrient content. ANOVA showed highly significant difference among genotype for all characters. The genotype had a wide range of Fe and Zn contents Velu *et al*. (2007) also recorded wide range in within population for micronutrients has also been reported for numerous other crops for instance in maize (Benziger and Long, 2000), sorghum (Reddy *et al*., 2005) and sesame (Pandey *et al*., 2016). Wide variation was also observed for seed yield and its other component traits under study among genotype. Thus the variability of wide range of variation for both kinds of nutritional and productive traits indicated the scope of development of Fe & Zn rich genotype in high yielding background of sesame through the exploitation within population variability. The magnitude of heritability was comparable for almost all the characters except for grain yield. The implies, low environmental influence and predominant role of genetic factors on the expression of Fe and Zn due to comparable genotypic and phenotypic variance while significant effect of environment was observed on grain yield. Aruselvi *et al*. (2007) in a study of 63 hybrids also observed higher heritability estimate for Fe and Zn. In the present investigation availability of substantial genetic variability for seed Fe and Zn coupled with high heritability (65-80%) indicated good project for solution of Fe and Zn rich genotype.

**Correlation analysis**

Significant and positive correlation was observed between Fe and Zn (Table) suggested that simultaneously component in likely to be highly effective. Highly significant correlation between Fe and Zn was earlier by (diapari *et al*, 2014) in chickpea. There was no correlation between seed yield and Fe and Zn contents in both the population indicating simultaneous selection foe Fe and Zn can be accomplished without compromising on seed yield. With Fe and Zn content in both the population indicated breeding for higher level of micronutrients without compromising the improvement for large grain size. And Zinc negatively correlated with days to 50% flowering, days to maturity and seed yield per plant. Highly significant positive phenotypic correlation coefficients was observed between seed yield/plant and plant height, capsules/plant and 1000 seed weight The results agreed with earlier Works by Sumathi and Murlidharan (2010). Khan *et al*., (2001), Uzun and Cagirgan (2001) and Sumathi *et al*., (2007) and Subramanian and Subramanian (1990). This emphasized that selection for higher Fe content would like to improve Zn also through correlated response in positive direction and 1000 seed weight in negative direction. The present findings of highly positive correlation between Fe and Zn find support Morgounov *et al*., 2007 in wheat. Correlation coefficient analysis is an important estimate in the determination of most effective statistics. Component breeding becomes simple task when there is a positive association of important characters. But when the characters are negatively associated, it would be difficult to exercise simultaneous selection for them in restructuring a variety. It is important to improve the micro-elements along with augmenting productive of crops. Some of the nutrients like Fe and Zn, obviously had a good impact on human nutrition. In fact both the elements to be positively associated and simply by conventional breeding program, it is possible to Fe and Zn content in seeds following simple breeding program. Among the morphological traits, seed yield was correlated positively with number of capsules/plant. Thus it is possible to increase seed yield further by augmenting this trait. Interestingly, Fe and Zn content were not correlated to either of the traits, thus restricting of plant type with high yield with higher content of Fe and Zn are achievable targets through simple breeding approach like transfer of gene for high Fe and Zn content to high yielding genotypes.

**PCA analysis**

Principal Component Analysis measures the importance and contribution of each component to total variance. It can be used for measurement of independent impact of a particular trait to the total variance whereas each coefficient of proper vectors indicates the degree of contribution of every original variable with which each principal component is associated. In present investigation, Principal component analysis has shown the genetic diversity of 50 genotypes following the Proportion of Variance Criterion by O'Rourke and Hatcher. According to this criterion, four principal components with cumulative variance of 66.93% (Table no: 4). which will provide a prominent idea of structure underlying the variables analyzed. For this study the first four axes obtained with Eigen value of > 1.0; which indicates that the identified traits within the axes exhibited great influence on the phenotype of the germplasm set. PCA1 accounted for 19.321 % of total variation in the population having the contribution from pH followed by Capsule per Plant, Seed Yield per Plant, 1000 Seed Weight and Zinc. Hence, the first component has phenological and yield related variables similar finding was reported by Sanni *et al.* (2008). Second Principal component accounts for 15.013% of total variation having major contribution of the traits like iron, zinc, Capsule per Plant and Seed Yield per Plant. Hence, the second component has micronutrient and agronomic traits. PCA 3 was accounted for 12.81% of total variation and the traits contribute most are capsule length, capsule breadth, primary branches and 1000 seed weight. PCA 5 was accounted for Days to 50 % Flowering, Days to Maturity and Capsule per Branch. PCA 4 was accounted for 10.213% of total variation and the having more contribution of the characters like Primary Branches, Secondary Branches and Days to 50% Flowering. Hence, from the current investigation the five three principal components contributing about half of the variance were plotted to observe relationships between the measured traits. The scree plots (Figure. 1) of PCA describe for first four eigen values and correspond to the whole percentage of the variance observed in the dataset.

**Table 1. List of germplasm**

| S.NO. | NAME | Land race/ High yielding Varieties (HYV) | S.NO. | NAME | Land race/ High yielding Varieties (HYV) |
|---|---|---|---|---|---|
| 1 | RT348 | HYV | 26 | IC – 204063 | LOCAL LAND RACE |
| 2 | TMV 4 | HYV | 27 | EC – 310448(36) | Exotic variety, Bulgaria |
| 3 | UMA | HYV | 28 | EC – 335004 | Exotic variety, Bangladesh |
| 4 | CUMS 04 | HYV | 29 | EC -334973(38) | Exotic variety, Bangladesh |
| 5 | RAMA | HYV | 30 | EC – 335004(34) | Exotic variety, Bangladesh |
| 6 | GT2 | HYV | 31 | EC – 303433(17) | Exotic variety, USA |
| 7 | V12 | LOCAL LAND RACE | 32 | EC – 164966(50) | Exotic variety, USA |
| 8 | OSC-593 | HYV | 33 | EC – 164966(52) | Exotic variety, USA |
| 9 | V-10 | LOCAL LAND RACE | 34 | EC – 310448(39) | Exotic variety, Bulgaria |
| 10 | AMRIT | HYV | 35 | EC – 41923 –(49) | Exotic variety, Bangladesh |
| 11 | B9 | HYV | 36 | EC – 303432 | Exotic variety, USA |
| 12 | RT-54 | HYV | 37 | EC – 303439 | Exotic variety, Bulgaria |
| 13 | SAVITRI | HYV | 38 | EC-306451 | EXOGENOUS VARIETY |
| 14 | HUMRA | HYV | 39 | CUHY-57 | C.U DEVELOPED VARIETY |
| 15 | TILLOTAMA | HYV | 40 | CUHY-23 | C.U DEVELOPED VARIETY |
| 16 | NIC 8316 | HYV | 41 | CR-11 | C.U DEVELOPED VARIETY |
| 17 | NIRMALA | HYV | 42 | CUHY-24 | C.U DEVELOPED VARIETY |
| 18 | TKG 355 | HYV | 43 | CUMS-17 | C.U DEVELOPED VARIETY |
| 19 | IC – 131490 | LOCAL LAND RACE | 44 | CUHY-13 | C.U DEVELOPED VARIETY |
| 20 | IC – 14331 | LOCAL LAND RACE | 45 | CUHY-36 | C.U DEVELOPED VARIETY |
| 21 | IC – 14053 | LOCAL LAND RACE | 46 | CR-11A | C.U DEVELOPED VARIETY |
| 22 | IC – 43033 | LOCAL LAND RACE | 47 | CUHY-45 | C.U DEVELOPED VARIETY |
| 23 | IC – 204159 | LOCAL LAND RACE | 48 | CUMS-9 | C.U DEVELOPED VARIETY |
| 24 | IC – 152485 | LOCAL LAND RACE | 49 | CUHY-27 | C.U DEVELOPED VARIETY |
| 25 | IC – 96230 | LOCAL LAND RACE | 50 | CUMS-11 | C.U DEVELOPED VARIETY |

**Table 2. ANOVA: Analysis of Variance and measures of variability for grain Fe and Zn content and other agronomic traits**

| SOV | DF | Fe | Zn | DF 50% floweing | 1000 seed wt | Seed yield per plant |
|---|---|---|---|---|---|---|
| Season | 1(1) | 22.3 | 13.7 | 10.8 | 4.1 | 5148.4 |
| Rep/season | 2(2) | 25.7 | 87.3 | 19.2 | 2.9 | 153.6 |
| Progeny | 29(23) | 503.0** | 270.7** | 3.1** | 7.2** | 533.8** |
| Season*Progeny | 29(23) | 90.3* | 48.1* | 0.3 | 0.5 | 337.0** |
| Error | 58(46) | 43.2 | 24.5 | 1.1 | 1.2 | 135.2 |
| Mean | | 46.7 | 44.6 | 45 | 9.6 | 2.14 |
| Range | | 29.9-77.2 | 30.7-63.0 | 43-47 | 6.6-11.8 | 1.36-2.93 |
| Heritability % | | 63.3 | 64.8 | 42.0 | 60.8 | 9.08 |

*significant at 5% level of significance **significant at 1% level of significance

**Table 3. Correlation coefficient for micronutrient content with yield and yield related traits**

| | PH | PB | SB | DF | IL | DM | CP | CL | CB | SW | SYP | Fe | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PH | 1 | | | | | | | | | | | | |
| PB | .392* | 1 | | | | | | | | | | | |
| SB | -.106 | .107 | 1 | | | | | | | | | | |
| DF | .217 | .121 | -.004 | 1 | | | | | | | | | |
| IL | .266* | .389** | -.263* | .027 | 1 | | | | | | | | |
| DM | .150 | .210 | .168 | .015 | .182 | 1 | | | | | | | |
| CP | .469** | .190 | .062 | -.033 | -.080 | .043 | 1 | | | | | | |
| CL | -.148 | -.046 | -.225 | -.116 | -.016 | .061 | -.186 | 1 | | | | | |
| CB | .080 | .267* | -.198 | .322* | .091 | -.034 | -.129 | .292* | 1 | | | | |
| SW | .197 | .523** | -.142 | .038 | .214 | .195 | .194 | .061 | .355* | 1 | | | |
| SYP | .425** | .124 | .077 | .146 | -.067 | -.206 | .330* | -.119 | .159 | .248* | 1 | | |
| Fe | .195 | -.020 | -.185 | .192 | -.029 | .008 | .055 | -.322* | .154 | .057 | .173 | 1 | |
| Zn | .214 | .012 | .057 | -.031 | .076 | -.001 | .044 | -.141 | .085 | 0.47 | .126 | .267* | 1 |

PH= Plant height, PB= Primary Branches, SB= Secondary branches, DF = 50% flowering, IL= inter node length, DM = Days to maturity, CP= Capsule/ plant, CL = Capsule length, CB= Capsule breath, SW= 1000 seed wt, SYP = Seed yield per plant, Fe= iron, Zn= Zinc

**Table 4. Eigen value and percent of total variation and component matrix for the principal component axes**

| Parameter | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|
| PH | .707 | -.216 | -.026 | .006 | .083 |
| CP | .694 | .526 | -.218 | .236 | .016 |
| SYP | .682 | .596 | .033 | .172 | .095 |
| SW | .473 | .084 | .464 | -.130 | .213 |
| DM | -.432 | .086 | .231 | .255 | .427 |
| ZN | .470 | -.695 | -.080 | .110 | .182 |
| FE | .107 | -.595 | -.238 | .377 | .406 |
| CL | -.041 | .032 | .676 | -.267 | .274 |
| CB | .086 | -.118 | .638 | .245 | -.501 |
| PB | -.204 | .366 | -.419 | -.415 | .308 |
| SB | .301 | -.199 | .200 | -.637 | .250 |
| DF | -.295 | .288 | .278 | .444 | .470 |
| Eigen Value | 2.319 | 1.802 | 1.538 | 1.228 | 1.146 |
| Variability (%) | 19.321 | 15.013 | 12.817 | 10.231 | 9.551 |
| Cumulative (%) | 19.321 | 34.33 | 47.151 | 57.382 | 66.934 |

PH= Plant height, PB= Primary Branches, SB= Secondary branches, DF = 50% flowering, IL= inter node length, DM = Days to maturity, CP= Capsule/ plant, CL = Capsule length, CB= Capsule breath, SW= 1000 seed wt, SYP = Seed yield per plant, Fe= iron, Zn= Zinc

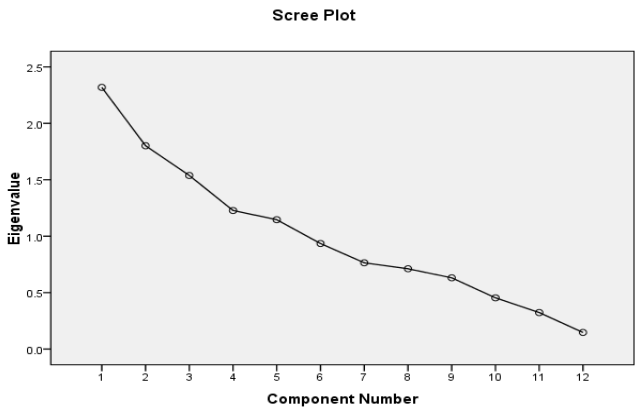


**Figure 1. Scree Plot**

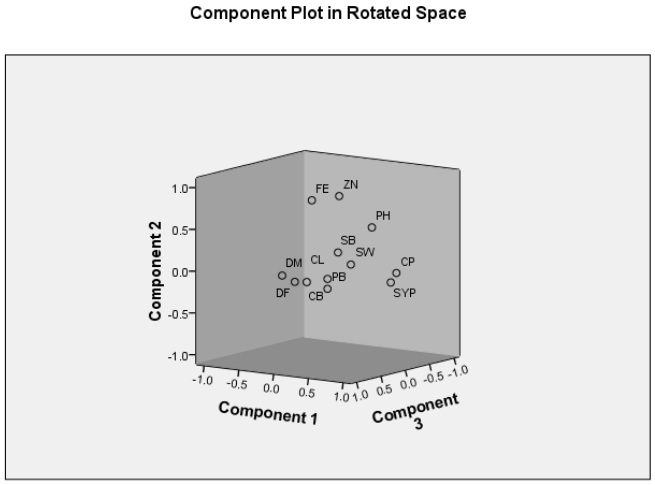


**Figure 2. Component plot in rotated space**

In rotated plot, (Figure 2), the variables are scattered across different components. The 3D rotation plot describes the variables which are grouped together have cumulative effect on the components.

The statistical properties of this interpretation have been described in detail by some researchers (Dehghani *et al*., 2008; Sabaghnia *et al*., 2011). Increased seed yield potential and micronutrient is an important goal for plant breeders and progress in yield potential results from the progressive accumulation of genes conferring higher yield or elimination of the unfavorable genes through the breeding process. The present investigation revealed that seed yield per plant had a strong relation with capsules per plant and plant height suggesting the need for more emphasis on these components for increasing the seed yield in sesame. Zinc and iron also had a strong relationship. Seed weight and branches had also strong relationship. The PCA may allow the plant breeder more flexibility in finding the number of plants to be evaluated and the plant breeder could use the multivariate methods by first determining the combination of traits that constitute an ideal plant. Here to increase seed yield per plant we have to give preference to capsules per plant and plant height. And to control any micronutrient either iron or zinc, by controlling only one element we can get our desired result. By plotting the PCAs that are considered to be important, plants close to the ideal plant would be selected (Yan and Rajcan, 2002). Thus the results of principal component analysis used in the study have revealed the high level of genetic variation existing in the population panel and explains the traits contributing for this diversity. Hence the results will be of greater benefit to identify parents for improving various morphological traits analyzed in this study. The PCA may be deemed important if their associated coefficients are of relative magnitude with breeding targets and given this apparent potential for using PCA, further work is required to compare multivariate methods for reaching actual gains.

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To

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**Diversity in sesame accessions**

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**Abstract:** Sesame known to be the most ancient oilseed crop in the world and well recognized for good quality edible oil due its high PUFA content, antioxidant properties, excellent nutritional and medicinal properties. The present investigation aims at analyzing the genetic diversity of 205 genotypes for 8 morphological characters. Phenotypic coefficients of variation exhibited a bit higher values but maintained a close relation with genotypic variation and genotypic co-efficient of variation for all the traits, indicating low G×E interaction. A combination of high heritability (broad sense) and high genetic advance indicate preponderance of additive gene action which is fixable. Additive gene action was prominent for the traits like no of capsules/plant, seed yiel /plant, and primary branches/plant. Hierarchical cluster analysis based on agro morphological traits results revealed that the inter cluster distance in most cases was larger indicating wider diversity among the germplasm of different groups. The maximum inter cluster distance was found between clusters V and III, followed by clusters V and II, clusters VI and II. The principal component analysis revealed that capsule number and 1000-seed weight had a strong relation with seed yield, suggesting the need for more emphasis on these components for increasing the seed yield in sesame.

**Key words:** genetic variability, hierarchical clustering, principal components, seed yield, sesame

## 1. Introduction

Sesame (*Sesamum indium* L.) is one of the most ancient and important oilseed crops known and used by mankind. Oil seeds constitute the major agricultural crop next only to food grains. Among the edible oilseed crops, sesame occupies a unique position as it can be grown throughout the year and moreover, it's poly-unsaturated fatty acid content makes it beneficial for human health. Sesame was cultivated and domesticated on the Indian subcontinent during Harappan and Anatolian eras around 4,000 years ago. (Bedigian and Van der Mesen, 2003).Due to the great stability of its healthy oil, easiness of extraction and resistance to drought, Sesame was very popular in the ancient world. It is considered as a nutritious oilseed crop being rich source of protein (18–25 %), carbohydrate (13.5 %), minerals and healthy polyunsaturated fatty acid (Bedigian et al. 1985). Sesame oil is favored as a media of cooking by Indians and Africans. Presence of sesamol, a unique anti-oxidant and more poly-unsaturated fatty acid, have made it to 'queen of oilseed crop' (Ashri 1998; Fukuda et al. 1986). Sesame ranks fifth for important edible oil crop in India after groundnut, rapeseed-mustard, sunflower and soybean. India holds first position in the world in sesame-acreage (24%) and contribution in export (40%) (FAO Statistics Division, 2010; Raikwar and Srivastva (2013). In spite of good production, productivity of sesame in India is low compared to other sesame producing countries. Other than favourable climate of production and remunerative price of the crop one of the simplest approach to increase horizontal expansion of the crop is to boost up productivity of the crop. Genetic up gradation of any crop primarily depends on utilization of existing genetic resource, and hence assessment of genetic diversity gets priority area. A large germplasm resource is always favoured in plant breeding program as many desirable traits may obviously remain in the population on which may be utilized in breeding program. It is difficult to maintain and evaluate large number of germplasm. The idea was to constitute novel variation from large gene pool and concept has been successfully established by Ellis et al (1998). The genetic improvement of any crop is

highly dependent on the magnitude of quite a few genetic parameters, such as, phenotypic variances and genotypic variances, phenotypic and genotypic coefficient of variation (PCV and GCV), broad sense heritability and genetic gain on which the breeding methods are designed for its further improvement. Assessing germplasm for diversity has an important implication in plant breeding program.

Different quantitative traits are usually pooled up together in multivariate analysis to reach towards a conclusive outcome of diversity based multivariate analysis is often used in selection of parents for hybridization program in different crops like horsegram (Dasgupta, 2005), blackgram (Dasgupta and Das, 1991, 1984) and sesame (Akbar et al 2011; Pham et al 2010). The present study has been conducted to assess the diversity of a core collection in sesame following morphological traits.

## 2. Materials and methods

Two hundred and five sesame genotypes collected from diverse eco-geographical regions of India and abroad., were planted in Randomized Block Design (RBD) with three replications having 10cm spacing between plants and 40cm between rows were studied during Summer 2015 at Agricultural Experimental Farm, University of Calcutta, Baruipur, West Bengal ($22^0 51^/$ N latitude and $88^0$ $24^/$ E longitude). Normal culture practices were followed during cultivation and irrigations were applied whenever the soils become very dry. All recommended package of practices were followed during the conduct of experiment. Observations were recorded on the basis of five random competitive plants selected from germplasm in every replication for seed yield and its attributing characters. The observations recorded on 8 morphological traits namely plant height (cm), days to 50% flowering, days to maturity, number of primary branches/plant, number of capsules/plant, capsule length (cm), 1000 seed weight and seed yield/plant. Statistical analysis was computed in SPSS 21.0 to cluster the genotypes based on genetic similarity.

Genotypic coefficient of variation, phenotypic coefficient of variation, heritability (broad sense) and genetic advance was measured by the methods described Burton (1968) and Hanson *et al.*, (1956). The PCA analysis reduces the dimensions of a multivariate data to a few principal axes, generates an Eigen vector for each axis and produces component scores for the characters Sneath et al (1973) and Ariyo and Odulaja (1991).

## 3. Results and Discussion :

### Components of genetic variability

Genotypes were significant for all traits as revealed from ANOVA. The estimates of GCV and PCV exhibited that PCV was greater than GCV for all traits indicating substantial role of environmental effect in the character expression (Table 1). Highest PCV was recorded by capsules per plant (56.5.) followed by number primary branches/plant (47.6) as well seed yield per plant (41.1). Conversely, moderate to low GCV and PCV was observed for the traits, plant height, days to 50% flowering, days to maturity and capsule length (Table 1). The broad sense heritability estimates were found to be higher for 1000 seed weight (88.1%), seed yield/plant (74.4%), number of capsule per plant (65.15%), plant height (60.60%), while capsule length (52.15%), days to flowering (51.57%) and days to maturity (31.99%) exhibited moderate heritability. Thus, in the present study, seed yield/plant, 1000 seed weight, number of branches/plant, number of capsules/plant, showed high GCV and heritability

Significant variability existed among the genotypes. Higher value of PCV than GCV indicated substantial role of environment in expression of the characters. Similar findings were reported by Reddy et al., (2001), Saha et al (2012), Tripathi et al (2013), Begum and Dasgupta (2014) and Iqbal et al (2015, 2016) and in sesame. In the present study, high GCV and PCV estimate for traits like seed yield/plant, 1000 seed weight, number of branches/ plant number of capsules per plant and plant height suggested sufficient variability of these characters and genetic

improvement through selection of these traits seemed to be possible. High GCV coupled with high heritability can provide more desirable information than a single parameter alone (Saha *et al*., 1990). In the present study, 1000 seed weight, seed yield/plant, number of capsule per plant, plant height exhibited higher heritability, while moderately high heritability was observed for capsule length, days to flowering, days to maturity. Similar results were reported by Parameshwarappa *et al*. (2010) in sesame for branches per plant, days to maturity, capsule length and capsules per plant. The estimates of heritability (broad sense) include both additive and non-additive gene effect and its higher estimates in broad sense indicates that the trait is least influenced by environmental effects. Traits with high heritability estimates can be utilized for genetic improvement as they have potential for large genetic determination (Vasline et al 2000). In fact, in present study characters like for 1000 seed weight, seed yield/plant, number of capsules per plant and plant height would hopefully leads towards similar repeatable results and hence more predictable.

**Cluster analysis**

Hierarchical cluster analysis based on agro morphological traits revealed that the inter cluster distance in most cases was larger indicating wider diversity among the germplasm of different groups. Cluster I included maximum number of genotypes (45) followed by cluster IV (25), cluster XII (24), cluster IX and cluster VII in such a way that genotypes having minimum genetic distance were grouped in same cluster and *vice versa*. Rest of the clusters was composed of mono-genotypic one. The range of inter cluster distance was 21.24 to 177.47, respectively (Table 3). The maximum inter cluster distance was found between clusters V and III (177.45), followed by clusters V and II (172.95), clusters VI and II (156.51), clusters V and X (153.09). The minimum inter cluster distance was recorded between clusters IV and XII followed by clusters 1 and IX (22.35). Cluster means of germplasm for eight characters (Table 3) revealed that cluster

XIII had maximum plant height and 1000 seed weight. Cluster III reported to be early maturing average plant type. Cluster V had maximum number of capsule per plant (Table 2). Highest seed yield per plant were recorded in cluster VII (Table 2).

Hierarchical cluster analysis grouped the genotypes into eleven clusters. The composition of clusters showed that clustering of germplasm was not associated with the geographical distribution and accessions were mainly grouped due to their morphological differences. Thus geographical isolation is not the only factor causing genetic diversity in sesame rather forces other than geographical origin such as genetic drift, natural and artificial selection, exchange of breeding material might have played an important role in the fixation of diversity among the germplasm lines (Kandamoorthy and Govindarasu 2005, Senapati and Sarkar 2005, Sabesan *et al.* 2009, Banumathy *et al.* 2010). A few ecological conditions could also direct the gene flow between populations from diverse geographical origins. The characters contributing maximum divergence needs greater emphasis for deciding on the clusters for the purpose of selection of parents in the respective cluster for hybridization and parents for hybridization should be selected from cluster having high intercluster distance like between cluster V and III or II.

In order to know with which combination type of agronomic traits the sesame would attain high grain yield, PCA was performed (Table 2). The Scree plot of the PCA (Figure 1) showed that the first three eigen values were correspond to the whole percentage of the variance in the dataset. The first three main PCAs are extracted from the complicated components, the total cumulative variance of these three factors amounted to 56.07% and these components had eigenvalues >1. The PCA simplifies the complex data by transforming the number of associated traits into a smaller number of variables as PCAs. The first PCA accounts for maximum variability in the data with respect to succeeding components. The PCA grouped the estimated sesame variables into three main components which PCA1 accounted for about 26.48% of the

variation; PCA2 for 16.56% and PCA3 for 13.06% (Table 2). The first PCA was related to capsule number and its contributing traits such as branch number and plant height, whereas the second PCA was related to days to 50% flowering and its contributing traits such as days to maturity (Table 4).

The traits, which contributed more positively to PCA1, were number of capsules / plant, number of branches/ plant, plant height, and seed yield per plant suggesting that this component reflected the yield potential of each genotype through some yield component aspects. In addition, the traits, which contributed positively to PCA2, were seed yield per plant, capsule length and thousand seed weight suggesting that this component reflected the yield potential of each genotype. In addition, the traits, which contributed positively to PCA3, were days to maturity, days to flowering, capsule length and seed yield per plant.

The PCA may allow the plant breeder more flexibility in finding the number of plants to be evaluated and the plant breeder could use the multivariate methods by first determining the combination of traits that constitute an ideal plant. By plotting the PCAs that are considered to be important, plants close to the ideal plant would be selected (Yan and Rajcan, 2002). The PCA may be deemed important if their associated coefficients are of relative magnitude with breeding targets and given this apparent potential for using PCA, further work is required to compare multivariate methods for reaching actual gains.

The PCA revealed that emphasis should be given to the traits like 1000 seed weight, branches/plant , plant height and capsules/plant during selection programme.

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**Table.1. Components of genetic variability in sesame.**

| S. No. | Character | Mean | GCV(%) | PCV(%) | h2 (%) | GA | GA as %of mean |
|---|---|---|---|---|---|---|---|
| 1 | Plant height | 87.93 | 19.85 | 25.20 | 62.06 | 22.31 | 8.12 |
| 2 | No. of branches per plant | 5.30 | 42.9 | 47.6 | 27.85 | 1.33 | 8.01 |
| 3 | Days to flowering | 41.84 | 6.81 | 9.51 | 51.27 | 3.00 | 2.40 |
| 4 | Days to maturity | 88.39 | 3.51 | 6.22 | 31.84 | 2.03 | 0.76 |
| 5 | No. of capsules per plant | 66.87 | 47.21 | 56.77 | 69.15 | 44.98 | 21.96 |
| 6 | Capsule length | 2.33 | 12.17 | 16.94 | 51.62 | 0.30 | 4.29 |
| 7 | 1000-seed weight | 2.58 | 23.29 | 24.99 | 86.85 | 1.07 | 13.55 |
| 8 | Seed yield per plant | 4.95 | 36.08 | 41.81 | 74.46 | 2.76 | 19.24 |

**Table.2.** Components of genetic variability in sesame.

| | Cluster | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| Plant height | 86.50 | 42.00 | 65.57 | 72.33 | 90.67 | 134.78 | 77.00 | 107.25 | 107.83 | 62.78 | 85.52 | 90.88 | 126.67 |
| Days to 50% flowering | 42.23 | 57.00 | 41.67 | 41.24 | 44.00 | 42.55 | 41.75 | 42.43 | 42.40 | 41.87 | 40.84 | 41.29 | 40.50 |
| No. of branches/plant | 4.23 | 3.67 | 3.10 | 4.79 | 8.00 | 8.00 | 11.66 | 7.22 | 4.30 | 4.40 | 6.60 | 6.06 | 5.67 |
| Days to maturity | 87.03 | 121.33 | 86.74] | 89.33 | 88.00 | 90.78 | 88.75 | 88.87 | 89.33 | 86.92 | 89.92 | 87.44 | 88.88 |
| Capsule length | 2.24 | 1.63 | 2.15 | 2.52 | 1.90 | 2.50 | 2.16 | 2.37 | 2.28 | 2.39 | 2.30 | 2.40 | 2.38 |
| No. of capsules/plant | 41.44 | 31.00 | 17.40 | 62.88 | 193.00 | 152.33 | 157.83 | 116.91 | 47.65 | 42.56 | 97.71 | 73.00 | 78.96 |
| 1000 seed weight | 2.47 | 1.47 | 2.64 | 2.81 | 2.16 | 2.36 | 2.62 | 2.73 | 2.49 | 2.46 | 2.46 | 2.64 | 2.85 |
| Seed yield /plant | 4.07 | 3.51 | 3.24 | 5.21 | 2.22 | 5.73 | 7.43 | 6.20 | 4.04 | 4.98 | 5.83 | 5.42 | 5.58 |

**Table 3.** Inter cluster distance among the genotypes of sesame.

| Cluster | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | | | | | | | | |
| 2 | 59.039 | | | | | | | | | | | | |
| 3 | 31.913 | 46.629 | | | | | | | | | | | |
| 4 | 25.848 | 56.703 | 46.128 | | | | | | | | | | |
| 5 | 151.685 | 172.956 | 177.475 | 131.519 | | | | | | | | | |
| 6 | 121.070 | 156.511 | 151.800 | 109.157 | 60.183 | | | | | | | | |
| 7 | 117.073 | 136.699 | 141.232 | 95.346 | 38.342 | 58.221 | | | | | | | |
| 8 | 78.373 | 113.691 | 108.027 | 64.397 | 78.010 | 44.910 | 51.108 | | | | | | |
| 9 | 22.335 | 76.482 | 52.060 | 38.662 | 146.431 | 108.179 | 114.703 | 69.354 | | | | | |
| 10 | 23.766 | 44.523 | 25.405 | 22.603 | 153.094 | 131.389 | 116.417 | 86.716 | 45.413 | | | | |
| 11 | 56.447 | 87.225 | 82.935 | 37.308 | 95.575 | 73.583 | 60.969 | 29.065 | 54.909 | 59.795 | | | |
| 12 | 31.957 | 74.560 | 61.204 | 21.249 | 120.092 | 90.759 | 86.177 | 46.918 | 30.655 | 41.472 | 25.422 | | |
| 13 | 55.064 | 103.947 | 86.837 | 56.679 | 119.715 | 73.910 | 93.429 | 42.702 | 36.652 | 73.586 | 45.243 | 36.324 | |

**Table 4.** Loadings of PCA for the estimated traits of sesame

| **TRAITS** | **Component** | | |
|---|---|---|---|
| | **PC-1** | **PC-2** | **PC-3** |
| Capsule/plant | .863 | .164 | -.049 |
| Primary branch | .754 | .142 | -.230 |
| Seed yield/plant | .620 | -.215 | .353 |
| Plant height | .534 | .262 | -.355 |
| Days to flowering | -.065 | .637 | .386 |
| Capsule length | .279 | -.562 | .441 |
| Seed weight | .207 | -.495 | .124 |
| Days to maturity | .102 | .444 | .614 |
| **Eigen value** | 2.119 | 1.325 | 1.042 |
| **Percentage of variance** | 26.486 | 16.560 | 13.026 |
| **Cumulative perecentage** | 26.486 | 43.047 | 56.072 |

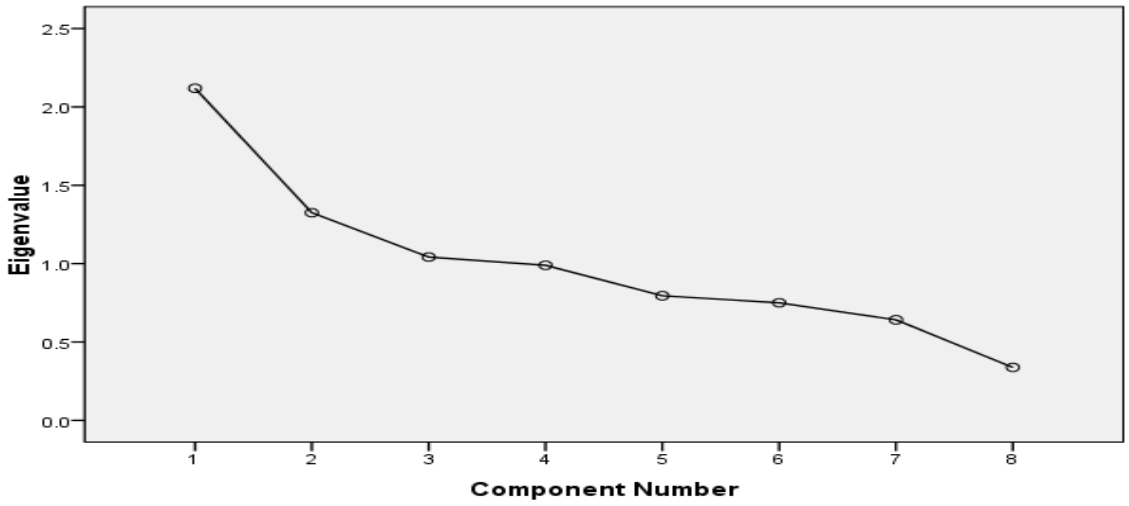


**Figure 1.** Scree plot showing eigenvalues

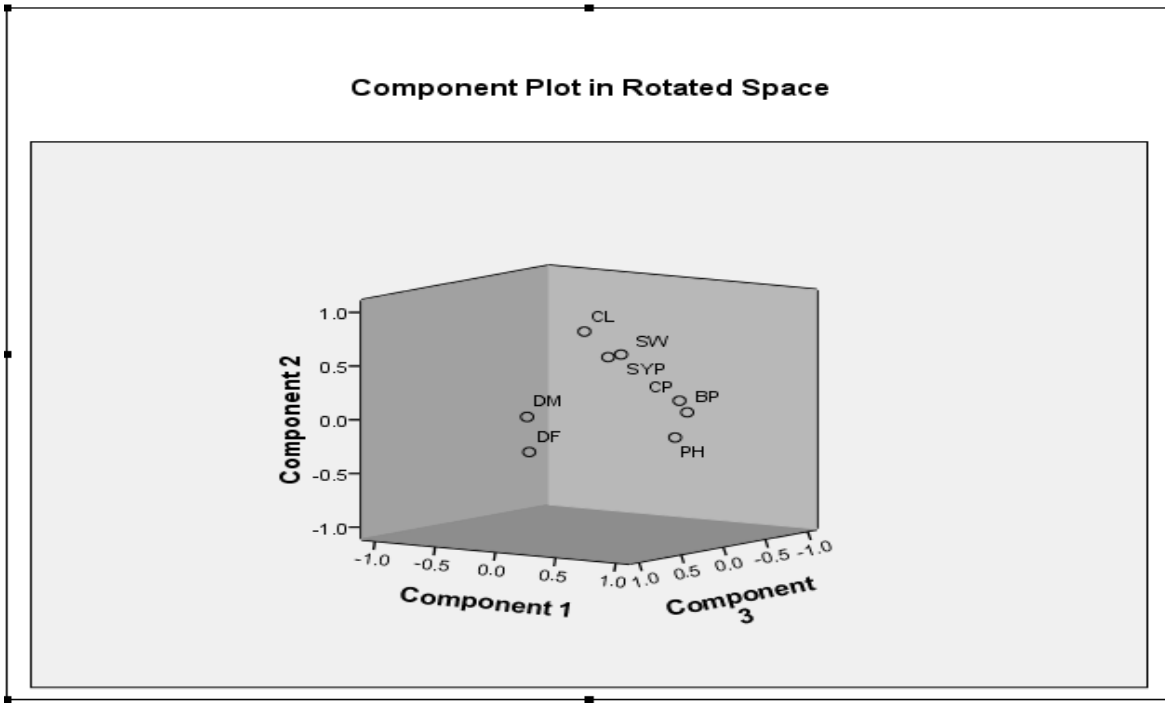


**Figure 2**. Plot of the first three PCAs showing relation among various sesame traits.