MOLECULAR AND CHEMO PROFILING OF GINGER (*Zingiber officinale* Rosc.) GENOTYPES

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BAGALKOT, KARNATAKA, INDIA

2018

MOLECULAR AND CHEMO PROFILING OF GINGER (*Zingiber officinale* Rosc.) GENOTYPES

Thesis submitted to the

University of Horticultural Sciences, Bagalkot

in partial fulfillment of the requirements for the award of the degree of

DOCTOR OF PHILOSOPHY

in

HORTICULTURE

By

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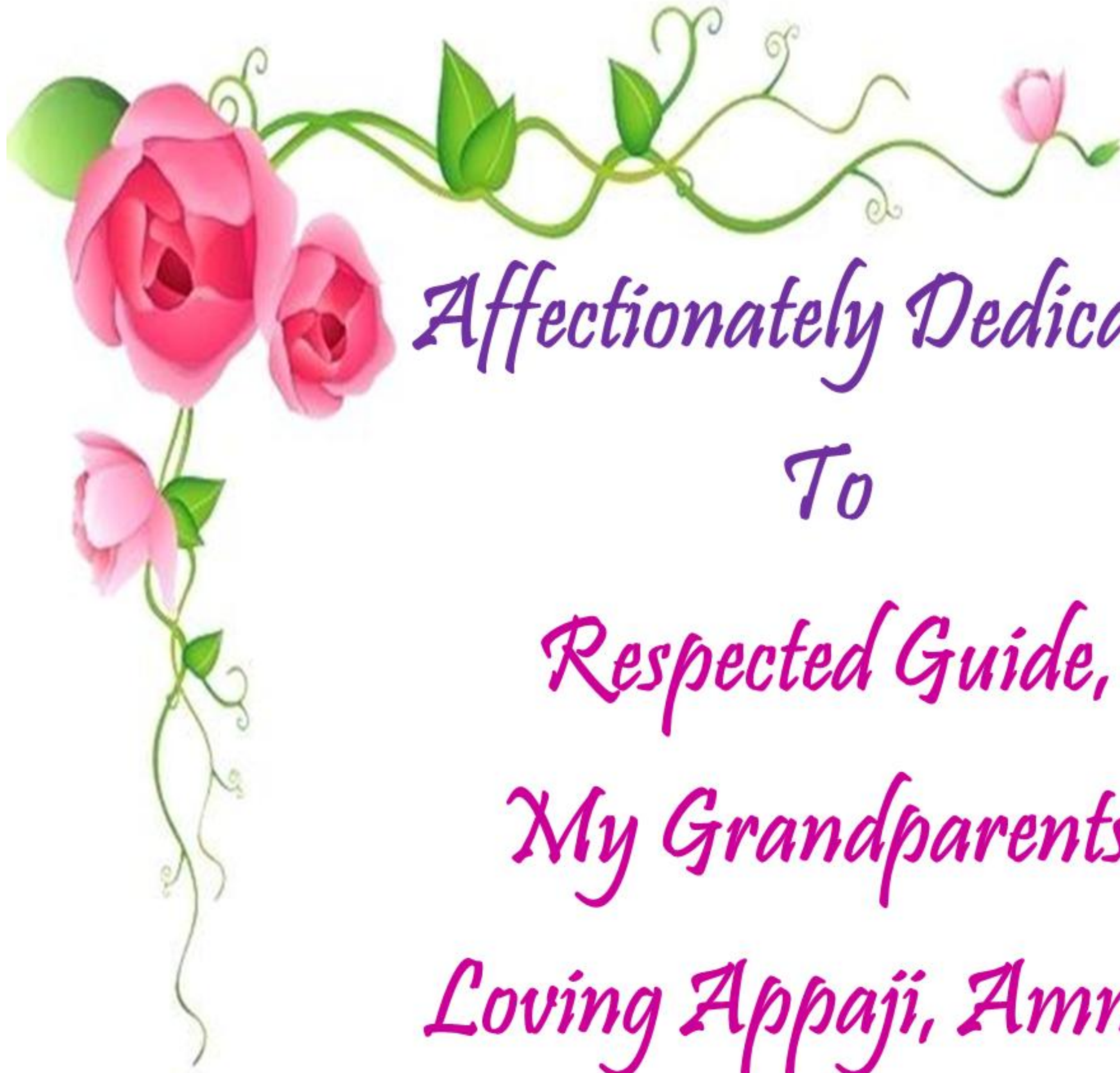

Affectionately Dedicated

To

Respected Guide,

My Grandparents,

Loving Appaji, Amma,

Bro, Sis in law, Master

Atharv, Athe, Mava

&

My Ever loving Husband

UNIVERSITY OF HORTICULTURAL SCIENCES, BAGALKOT
COLLEGE OF HORTICULTURE, BENGALURU
DEPARTMENT OF HORTICULTURE

CERTIFICATE

This is to certify that the thesis entitled **"MOLECULAR AND CHEMO PROFILING OF GINGER (*Zingiber officinale*Rosc.) GENOTYPES"** submitted by **Mrs. AKSHITHA, H. J., UHS12PGD43** for the degree of **DOCTOR OF PHILOSOPHY** in **HORTICULTURE** to the University of Horticultural Sciences, Bagalkot, is a record of *bona-fide*research work done by her during the period of her study at University of Horticultural Sciences, Bagalkot under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

Bengaluru
July, 2018

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ACKNOWLEDGEMENT

"Gratitude is the memory of the heart"

In the height of reaching a milestone in my life, I wish to remember and acknowledge all who made this possible and helped me in a constructive fashion.

*I wish to express my deep sense of gratitude and indebtedness to **Dr. K. Umesha,** Professor, Department of Plantation, Spices, Medicinal and Aromatic Crops, College of Horticulture, UHS Campus, Bengaluru and esteemed chairperson of my Advisory Committee for his scholarly guidance, valuable advice and constant encouragement.*

*With the sense of pride I would like to place on record my sincere thanks to **Dr. D. Prasath** Principal Scientist, ICAR-Indian Institute of Spices Research, Kozhikode and Co-chairperson of my advisory committee for his valuable suggestion and kind co-operation throughout the duration of this study. I will be indebted forever to him for his patience, constant support, guidance, editorial advice, constructive and critical review to bring the thesis at its present stage.*

*I would like to express my gratitude to **Dr. N. K. Leela**, Principal Scientist, ICAR-Indian Institute of Spices Research, Kozhikode and member of my advisory committee for her valuable suggestions and help rendered during the biochemical studies.*

*I would like to express my gratitude to **Dr. M. Shivapriya,** Assistant Professor, Department of Biotechnology and Crop Improvement, College of Horticulture, Bengaluru and member of my advisory committee for her valuable suggestions and ever willing help rendered during the thesis work.*

*My heartfelt thanks to **Dr. Maruthi Prasad**, Assistant Professor, Department of PMASC, College of Horticulture, Bengaluru and member of my advisory committee for his valuable suggestions and encouragement provided.*

*I am highly indebted to **Dr. M. Anandaraj,** Former Director, ICAR-IISR, Kozhikode for his critical inputs and all the help rendered during selection of research topic and permitting to carry out the research work at the institute.*

*Words fall short to express my deep sense of gratitude to **Dr. K. Nirmal Babu**, Director, ICAR-IISR, Kozhikode for his help, consistent encouragement and everlasting support which helped me in the*

successful completion of research and thesis work. I am thankful to him for providing all the facilities to carry out the research work in the institute.

I am also thankful to ***Dr. B. Sasikumar****, Former Head, Division of Crop Improvement and Biotechnology, ICAR-IISR, Kozhikode and* ***Dr. S. Devasahayam****, Former Head, Division of Crop Protection, ICAR-IISR, Kozhikode for all the support and encouragement during the course of work.*

I thank ***Dr. H. B. Lingaiha,*** *Vice Chancellor, UHS, Bagalkot and* ***Dr. Vishuvardhan****, Dean, College of Horticulture, Bengaluru for their help during course of study. I am also thankful to all the staff members of College of Horticulture, Bengaluru for all the help rendered.*

I express my sincere thanks to ***Dr. J. Rema****, Head, Division of Crop Improvement and Biotechnology;* ***Dr. Santosh J. Eapen****, Head, Division of Crop Protection;* ***Dr. Thankamani, C. K,*** *Head, Division of Crop Production and PHT, ICAR-IISR, Kozhikode for their help and co-operation during the course of my study.*

I am thankful to ***Dr. S. J. Ankegowda****, Principal Scientist and Head, ICAR-IISR Regional Station, Appangala for all the help and encouragement rendered.*

I express my sincere thanks to ***Dr. Lijo Thomas****, Scientist, ICAR-IISR, Kozhikode for helping me in getting the red ginger seed material and I thank* ***Mr. K. C. Cheriyan****, Ginger farmer from Kottayam for providing the seed material.*

My special thanks to my dear friends ***Mrs. S. Aarthi, Ms. R. Sivaranjani, Dr. R. Praveena, Dr. M. S. Shivakumar, Mr. Muhammed Nissar*** *who helped me a lot during the course of study.*

My special thanks to all the ***Scientific , Administrative and Technical staff of ICAR-IISR, Kozhikode*** *for all the help and support rendered during the course of study. I am thankful to all the labmates of Central facility and Biochemistry lab ICAR-IISR and special thanks to* ***Ms. Prashina, Ms. Aparna, Ms. Lijina, Mrs. Karthika and Mrs. Ann Jasmine.***

My special thanks to ***Dr. Mohammed Faisal****, Scientist and* ***Mr. Muraleedharan, P.,*** *AAO, ICAR-IISR RS, Appangala for their patience and help rendered during the course of reserach. My thank to* ***Dr. Alagupalamuthirsolai****,* ***Dr. Balaji Rajkumar****,* ***Dr. Narendra Chaudhary****,* ***Mr. Honnappa Asangi,*** *Scientists and all other staff of ICAR-IISR RS, Apapngala for all the help and support.*

I am thankful to ***Dr. V. Venkataravanappa****, Scientist, ICAR-CHES, Chettalli for all the help and support during course of study.*

*I am thankful to **Mr. Harish, B. T.,** Technician and all other staff of Experimental Farm, Peruvannamuzhi who helped me conducting the field experiments.*

*I am highly fortunate in having my dear and near friend **Veena, G. L.** whose hands were evident at every moment of tension, anxiety and achievements. I am really thankful to her. My heartfelt thanks to **Dr. H. C. Vikram** who helped me a lot for stastical analysis of data and also for all the support and encouragement.I convey my heartfelt thanks to all my **friends** and **my batchmates** for their help, consistent encouragement and everlasting support.*

*I submit all eulogize grandeur and reverence to my loving Grandma **Mrs. G. B. Gangamma,** my parents **Mr. Jagannath Gowda, H. B. and Mrs.Prema, B. G.,** my brother and sis-in-law **Mr. Abhishek, H.J., Mrs. Anushree, M. L. and Master Atharv,** my grandmother **G. B. Gangamma,** my everloving and caring husband **Mr. Muralidhara, B. M.,** my in laws **Mr. Munivenkate Gowda and Mrs. Lakshmidevamma** for their never ending support, affection and love. I express my wholehearted thanks to them for all they have done for me. I am also grateful to my uncles, aunties, brothers, sisters, sister in laws and all my relatives for their love, care and support.*

Finally, I thank the eternal love of the great Almighty for gracing the peaceful atmosphere during the course of my study.

.... any omission in this small manuscript does not mean lack of gratitude.

Bengaluru

July, 2018 *(AKSHITHA, H. J.)*

MOLECULAR AND CHEMO PROFILING OF GINGER (*Zingiber officinale* Rosc.) GENOTYPES

ABSTRACT

Studies on Molecular and chemo profiling of ginger (*Zingiber officinale* Rosc.) genotypes were carried out during 2016-17 and 2017-18 at ICAR-Indian Institute of Spices Research, Kozhikode and ICAR-IISR, Experimental Farm, Peruvannamuzhi, to know the morphological, biochemical and molecular diversity among 28 ginger genotypes, 1 *Curcuma* sp. and 1 *Kaempferia* sp. Among the 28 genotypes studied, highest projected yield was recorded in genotype Maran (17.71 t/ha) followed by Acc. 247 (16.33 t/ha) and Himachal (16.06 t/ha). Growth and yield parameters *viz.,* number of tillers per clump, total number of leaves, yield per plant, essential oil, oleoresin and crude fibre content exhibited high heritability coupled with high genetic advance as per cent mean which can be the reliable selection parameters for ginger crop improvement. Grouping of genotypes based on DUS descriptors showed narrow variability for most of the morphological characters, whereas rhizome characters exhibited remarkable variability. Chemical profiling showed that, Red ginger had the highest oleoresin and essential oil percentage of 12.18 % and 6.00 %, respectively. Among other ginger genotypes, Arunachal Pradesh local (8.55 %), Rio de Janeiro (7.77 %) and Acc. 65 (7.10 %) revealed high oleoresin content and genotype Arunachal Pradesh local had higher oil content (3.00 %). Zingiberene was the major component present in the essential oil of ginger genotypes and the highest content was observed in cultivar 'Maran'. Molecular profiling of ginger genotypes by RAPD and SSR primers revealed that, the markers were efficient in clustering the other species *viz.,* mango ginger and black ginger but, in case of ginger genotypes irrespective of place of collection or origin, genotypes were grouped into different clusters. Grouping was not on the basis of any morphological, yield or biochemical characters. Identification of SNPs using comparative transcriptome was carried out that can be further utilized to identify the genotype specific markers.

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Student

(K. Umesha)
Chairperson

ಶುಂಠಿ (*ಜಿಂಜಿಬರ್ ಅಫಿಷಿನಾಲಿ ರೋಸ್*) ತಳಿಗಳ ಆಣ್ವಿಕ ಮತ್ತು ಜೀವರಾಸಾಯನಿಕ ಪ್ರೋಫೈಲಿಂಗ್

ಪ್ರಬಂಧದ ಸಾರಾಂಶ

ಶುಂಠಿ ತಳಿಗಳ ಆಣ್ವಿಕ ಮತ್ತು ಜೀವರಾಸಾಯನಿಕ ಪ್ರೋಫೈಲಿಂಗ್ ಅಧ್ಯಯನವನ್ನು 28 ಶುಂಠಿ ತಳಿಗಳು, 1 ಕರ್ಕುಮ ಸ್ಪೀಸಿಸ್ ಮತ್ತು 1 ಕೆಂಫೆರೀಯಾ ಸ್ಪೀಸಿಸ್‌ಗಳ ನಡುವಿನ ಸಸ್ಯ ಸ್ವರೂಪ, ಜೀವರಾಸಾಯನಿಕ ಮತ್ತು ಆಣ್ವಿಕ ವೈವಿಧ್ಯತೆಯನ್ನು ತಿಳಿಯಲು, ಭಾ.ಕೃ.ಅ.ಪ.–ಭಾರತೀಯ ಸಂಬಾರ ಬೆಳೆಗಳ ಸಂಶೋಧನ ಸಂಸ್ಥೆ, ಕೋಯಿಕ್ಕೋಡ್ ಮತ್ತು ಭಾ.ಕೃ.ಅ.ಪ.–ಐ.ಐ.ಎಸ್.ಆರ್., ಪ್ರಾಯೋಗಿಕ ಕ್ಷೇತ್ರ, ಪೆರುವನ್ನಮುಳಿಯಲ್ಲಿ 2016–17 ಮತ್ತು 2017–18 ರಲ್ಲಿ ಕೈಗೊಳ್ಳಲಾಯಿತು. ಅಧ್ಯಯನ ನಡೆಸಿದ 28 ಶುಂಠಿ ತಳಿಗಳ ಪೈಕಿ ಅತ್ಯಧಿಕ ಯೋಜಿತ ಇಳುವರಿಯನ್ನು ಮಾರನ್ (17.71 ಟನ್/ಹೆ) ತಳಿಯು ನೀಡಿತು ಮತ್ತು ಅತಿ ಹೆಚ್ಚು ಇಳುವರಿ ನೀಡಿದ ಇತರ ತಳಿಗಳೆಂದರೆ Acc. 247 (16.33 ಟನ್/ಹೆ), ಹಿಮಾಚಲ್ (16.06 ಟನ್/ಹೆ) ಮತ್ತು ಐಐಎಸ್‌ಆರ್–ವರದ (15.85 ಟನ್/ಹೆ). ಬೆಳವಣಿಗೆ ಮತ್ತು ಇಳುವರಿ ನಿಯತಾಂಕಗಳಾದ, ಪ್ರತಿ ಗಿಡದಲ್ಲಿನ ಕಂದುಗಳ ಸಂಖ್ಯೆ, ಒಟ್ಟು ಎಲೆಗಳ ಸಂಖ್ಯೆ, ಪ್ರತಿ ಗಿಡದ ಇಳುವರಿ ಮತ್ತು ಗೆಡ್ಡೆಯಲ್ಲಿನ ತೈಲ, ಓಲಿಯೋರೆಸಿನ್ ಮತ್ತು ಕಚ್ಚಾ ನಾರಿನ ಅಂಶಗಳು ಉನ್ನತವಾದ ಅನುವಂಶಿಕತೆ ಮತ್ತು ಅದರೊಂದಿಗೆ ಅಧಿಕ ಶೇಕಡಾವಾರು ಅನುವಂಶಿಕ ಮುಂಗಡತೆಯನ್ನು ಪ್ರದರ್ಶಿಸಿದವು. ಈ ನಿಯತಾಂಕಗಳನ್ನು ಶುಂಠಿ ಬೆಳೆ ಸುಧಾರಣೆಗೆ ವಿಶ್ವಾಸಾರ್ಹ ಆಯ್ಕೆ ನಿಯತಾಂಕಗಳನ್ನಾಗಿ ಉಪಯೋಗಿಸಬಹುದು. DUS ವಿವರಣೆಗಳ ಆಧಾರದ ಮೇಲಿನ ತಳಿಗಳ ವರ್ಗೀಕರಣವು ಸಸ್ಯ ಸ್ವರೂಪ ಗುಣಗಳಿಗೆ ಕಿರಿದಾದ ವ್ಯತ್ಯಾಸವನ್ನು ಪ್ರದರ್ಶಿಸಿದರೆ ಗೆಡ್ಡೆಗಳ ಗುಣಗಳು ಗಮನಾರ್ಹ ವ್ಯತ್ಯಾಸವನ್ನು ಪ್ರದರ್ಶಿಸಿವೆ. ಕೆಂಪು ಶುಂಠಿಯು ಅತ್ಯಧಿಕ ಶೇಕಡಾವಾರು ಓಲಿಯೋರೆಸಿನ್ (12.18 %) ಮತ್ತು ತೈಲ (6.00 %) ವನ್ನು ಹೊಂದಿದೆ. ಇನ್ನಿತರ ಶುಂಠಿ ತಳಿಗಳಲ್ಲಿ, ಹೆಚ್ಚು ಓಲಿಯೋರೆಸಿನ್ ಅಂಶವನ್ನು ಅರುಣಾಚಲ ಪ್ರದೇಶ ಲೋಕಲ್ (8.55 %), ರಿಯೋ ಡಿ ಜನೈರೊ (7.77 %) ಮತ್ತು Acc. 65 (7.10 %) ಹೊಂದಿವೆ. ಹಾಗೆಯೇ ಹೆಚ್ಚು ತೈಲದ ಅಂಶವನ್ನು ಅರುಣಾಚಲ ಪ್ರದೇಶ ಲೋಕಲ್ (3.00 %) ತಳಿಯು ಹೊಂದಿದೆ. ಶುಂಠಿಯ ತೈಲದಲ್ಲಿ ಕಂಡುಬಂದ ಪ್ರಮುಖ ಅಂಶವಾದ ಜಿಂಜಿಬೆರಿನ್ ಮಾರನ್ ತಳಿಯಲ್ಲಿ ಹೆಚ್ಚಿನ ಪ್ರಮಾಣದಲ್ಲಿ ಕಂಡುಬಂದಿದೆ. RAPD ಮತ್ತು SSR ಪ್ರೈಮರ್‌ಗಳನ್ನು ಉಪಯೋಗಿಸಿ ಮಾಡಿದ ಆಣ್ವಿಕ ಪ್ರೋಫೈಲಿಂಗ್‌ನಿಂದ ಕಂಡುಬಂದದ್ದೆಂದರೆ, ಪ್ರೈಮರ್‌ಗಳು ಮಾವಿನ ಶುಂಠಿ ಮತ್ತು ಕಪ್ಪು ಶುಂಠಿಯನ್ನು ಬೇರೆ ಗುಂಪುಗಳನ್ನಾಗಿ ಬೇರ್ಪಡಿಸುವಲ್ಲಿ ಸಮರ್ಥವಾಗಿವೆ; ಆದರೆ, ಇತರ ಶುಂಠಿ ತಳಿಗಳ ವರ್ಗೀಕರಣ, ತಳಿ ಸಂಗ್ರಹಿಸಿದ ಸ್ಥಳದ ಮೇಲಾಗಲಿ ಅಥವಾ ಸಸ್ಯ ಸ್ವರೂಪ, ಇಳುವರಿ ಮತ್ತು ಜೀವರಾಸಾಯನಿಕ ಗುಣಗಳ ಮೇಲಾಗಲಿ ಆಧಾರಿಸಿಲ್ಲ. ತುಲನಾತ್ಮಕ ಟ್ರಾನ್ಸ್ಕ್ರಿಪ್ಟೋಮ್ ಅನ್ನು ಬಳಸಿಕೊಂಡು SNP ಗಳನ್ನು ಗುರುತಿಸಲಾಗಿದೆ, ಈ SNP ಗಳನ್ನು ತಳಿಗಳಿಗೆ ನಿರ್ದಿಷ್ಟ ಮಾರ್ಕರ್‌ಗಳನ್ನು ಗುರುತಿಸಲು ಪ್ರಯೋಜನಕಾರಿಯಾಗಿವೆ.

(ಅಕ್ಷಿತ, ಹೆಚ್. ಜೆ.)
ವಿದ್ಯಾರ್ಥಿ

(ಕೆ. ಉಮೇಶ)
ಮುಖ್ಯ ಸಲಹೆಗಾರರು

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I. INTRODUCTION

Ginger of commerce is the underground rhizome of *Zingiber officinale* Rosc. (2n=22), belonging to the family Zingiberaceae is originated from South-East Asia. It is one of the oldest and most important spices, being cultivated in Tropical Asia for over 3000 years. It is one of the earliest oriental spices known to Europe and is still in large demand today. The rhizomes may be scraped or peeled before drying and are esteemed for their aroma, flavour and pungency. It may also be used in powdered form (Purseglove *et al.*, 1981).

It is a herbaceous perennial, but cultivated as an annual, with crop duration of seven to ten months. Major active compounds present in ginger are gingerol, shogaol and zingerone. It is propagated vegetatively by rhizomes.

India is the largest producer of ginger in the world. During the year 2015-16, it was cultivated on an area of 1,61,670 ha with a production and productivity of 10,87,560 MT and 6.7 MT/ha, respectively (DASD, 2016). Top five ginger producing countries are India, China, Nepal, Nigeria and Thailand (FAOSTAT, 2014). Major importing countries are Japan, United States, United Kingdom, Saudi Arabia, Singapore and Malaysia (Anne Plotto, 2002).

Ginger has been used as medicine from vedic period and is called “Maha aushadhi” (Sanskrit), means the great medicine. Rhizome of ginger has been used as a medicine in Chinese, Indian and Arabic herbal traditions since ancient times (Atman and Marcussen, 2001). In traditional medicine, it is used as a carminative or antiflatulent. Ginger is a pungent, aromatic stimulant, much employed as a stomachic in flatulency and spasm of the stomach and bowels. Ginger contains biologically active constituents including the main pungent principles, the gingerols and shogaols. The Greek physician Galen described ginger as a purificant of body (Langner *et al.*, 1998).

Largest collection of ginger germplasm (675 accessions) is being conserved at ICAR-Indian Institute of Spices Research, Kozhikode, Kerala which is also NAGS centre of ginger.

Several cultivars of ginger are grown in different ginger growing areas of India and they are generally named after the localities where they are grown. Some of the prominent indigenous cultivars are Maran, Kuruppampadi, Ernad, Wayanad, Himachal and Nadia. The exotic cultivar ‘Rio-de-Janeiro’ have also become very popular among cultivators. There are about 9 improved varieties of ginger developed from different institutes (Jayashree *et al.,* 2015). Among the improved varieties, the variety IISR Varada is suited for fresh ginger, dry ginger and making candy while, varieties Athira, Karthika and Aswathy from KAU are rich in essential oil.

Breeding of ginger through selection and hybridization is seriously handicapped by lack of variability, absence of natural seed set and exclusive vegetative propagation. Landraces are known by place of domestication and all of them may not be distinct genetically. In ginger there is no sexual reproduction. Geographical spread accompanied by genetic differentiation into locally adopted population augmented by mutation is the main factor for diversity (Singh *et al.*, 2012). The knowledge of the variability structuring could allow not only the description of genotypes but also development of a conservation strategy for future breeding purposes.

Most of the varieties have vernacular names and as the crop is propagated vegetatively, chances of mixing are more. Generally, ginger genotypes are identified based on morphological traits. But the assessment of these traits is difficult and their evaluation can be subjective considering that most of these cultivars are related. This identity of the cultivars has lead to considerable confusion in the gene pool, as it is often observed that a particular genetic entity is known by different names at different places and they bear separate accession number in the gene bank leading to duplication. This can lead to considerable problems in bioprospecting of the crop. Further, most of the ginger cultivars are not easily differentiated based on rhizome or aerial morphological features, further confounding the confusion to a greater extent.

The knowledge of extent of genetic variability in the population is required for further improvement of the crop. So in this regard studies on genetic variability, heritability will be helpful in identifying the particular traits in ginger which can be further utilized in the crop improvement programs.

Fibre, volatile oil and pungency level are the most important criteria in assessing suitability of ginger rhizomes for particular processing purposes. These quality components vary in fresh and dried forms of ginger and also at different maturity stages. Hence the biochemical characterization is helpful for identifying the varieties for different end uses.

The molecular approach for identification of plant varieties/genotypes seems to be more effective than the traditional morphological markers because it allows direct access to the hereditary material and makes it possible to understand the relationships, between plants (Williams *et al.*, 1990 and Paterson *et al.*, 1991).Molecular marker technology is the powerful tool for determining genetic variation in ginger genotypes as they can reveal abundant difference among genotypes at the DNA level, providing a more direct, reliable and efficient tool for germplasm characterization, conservation, management and untouched by environmental influence.

Hence, in the present study, a combination of morphological, biochemical and molecular techniques are proposed to characterize ginger genotypes with following objectives.

- Genetic diversity analysis of local genotypes and released varieties of ginger based on DUS characters for identifying superior genotypes
- Biochemical profiling of local types and released varieties of ginger based on essential oil, oleoresin and crude fibre for identifying varieties for different end use
- DNA profiles based diversity analysis

II. REVIEW OF LITERATURE

Zingiberaceae is the largest family in the order Zingiberales with 50 genera and over 1,500 species distributed mainly in tropics and subtropics. India has rich diversity of Zingiberaceous plants, perhaps 200 of the world taxa occur in India. Many species are economically important as source of food (Anon., 2017).

Classification of the Zingiberaceae family was first proposed in 1889 and refined by others. Since then four tribes recognized (Globbeae, Hedychieae, Alpinieae and Zingibereae) based on morphological features, such as number of locules and placentation in the ovary, development of staminodia, modifications of the fertile anther and rhizome-shoot-leaf orientation.

Kress *et al.* (2002) used phylogenetic investigation and proposed new classification of the Zingiberaceae that recognizes four sub families and four tribes: Siphonochiloideae (Siphonochileae), Tamijioideae (Tamijieae), Alpinioideae (Alpinieae, Riedelieae) and Zingiberoideae (Zingibereae, Globbeae). This classification was done by phylogenetic analyses based on DNA sequences of the nuclear internal transcribed spacer (ITS) and plastid matK regions.

Many species under Zingiberaceae are important ornamental plants, spices and medicinal plants. Commercial Zingiberaceous spices traded are small cardamom, large cardamom, ginger and turmeric (Singh *et al.*, 2012). Genetic diversity analyses in these species are done by morphological, biochemical and molecular techniques.

Ginger is not known to occur in truly wild state, but was under cultivation from ancient times in India as well as in China (Bailey, 1949). There is no definite information on the primary center of domestication. Because of the easiness with which ginger rhizomes can be transported to long distances, it has spread throughout the tropical and sub-tropical regions in both hemispheres. The earliest mention of ginger cultivation in history is probably by Rabbi Benjamin Tudella, who traveled between 1159 and 1173 A.D. and gave an account of spices grown on the west coast of India. Tudella gives a vivid description of the place and trade in spices as well as cultivation of spices in and around the ancient port of Quilon in the State of Kerala (Mahindru, 1982). Marco Polo (A.D.1298), in his famous travelogue, writes: "good ginger also grows here and is known by the name of “Quilon ginger”. Ginger is indeed the most widely cultivated spice (Lawrence, 1984).

Characteristic features of ginger

- Herbaceous perennial grown as annual crop
- Plant is erect has many fibrous roots, aerial shoots (pseudostem) with leaves and the underground stem (rhizome)
- Leaves are isobilateral, upper epidermal cells of leaf are polygonal and predominantely elongated at right angle to the long axis of the leaf.
- Flowers are produced in pedicled spikes arising directly from the rhizomes. Oval or conical spike consists of overlapping bracts, from the axil of which flowers arise.
- Fleshy sympodial rhizome is hard and thick.

Ravindran *et al.* (2005)

In this chapter the reviews on studies carried out to characterize ginger genotypes by morphological, biochemical and molecular techniques are presented.

2.1 Morphological characterization

2.1.1 Variability

Variability of yield and yield attributing characters are reported in ginger (Khan, 1959; Thomas, 1966; Krishnamurthy *et al.*, 1972; Muralidharan and Kamalam, 1973; Mohanty and Sharma, 1979; Nybe and Nair, 1979; Kumar *et al.*, 1980; Mohanty *et al.*, 1981; Arya and Rana, 1990; Sasikumar *et al.*, 1992; Ravindran *et al.*, 1994; Sasikumar *et al.*, 1994; John and Ferreira, 1997; Chandra and Govind, 1999; Yadav, 1999; Singh *et*

al., 1999; Singh *et al*., 2000; Gowda and Melanta, 2000; Mohandas *et al*., 2000; Singh, 2001; Tiwari, 2003; Abraham and Latha, 2003; Rana and Korla, 2007 and Lincy *et al*., 2008).

Among the 20 indigenous and exotic varieties of ginger evaluated for yield by Saikia and Shadeque (1992), significant variations in yield were observed. Among the varieties, Nadia yielded the highest (6.7 t ha^{-1} fresh ginger) followed by Chekerella (5.7 t ha^{-1}).

Sasikumar *et al.* (1992) evaluated one hundred ginger genotypes for plant height, leaf number, tiller number, leaf length and width, days to maturity, dry recovery and yield $plant^{-1}$. Good variability was observed for number of tillers and yield $plant^{-1}$. Among the genotypes, Santhing Laidum, Sangiguda, PGS-37, PGS-39, Maran, Acc.506 and Himachal recorded >600 g (mean) fresh rhizome yield $plant^{-1}$. Dry recovery ranged from 19 % (PGS-37) to 25 % (Acc. 506) and days to maturity from 214 (PGS-39) to 223 (Himachal) days. Study also indicated that, types like China, Ernad, Chernad, Kuruppambadi Local, Thing Pui, Zahirabad, Acc. 243, Mowshom, Kadan Nariampara, S-557 and Assam are quite suitable for conversion to dry ginger as the dry recovery percentage of these types was above 25 %.

Pandey and Dobhal (1993) evaluated 29 ginger genotypes. The collection H-85 (203.2 g) followed by NH 6/4 (175.6 g) and MNCH I56 (174.3 g) were the high yielders and also exhibited higher mean values for weight of fingers and primary rhizome. The genotypes BD-16 (34.7 cm) and DKH-34 (33.1 cm) were taller while the genotypes DKH-28 (7.10) and TURA (7.95) exhibited higher number of tillers $plant^{-1}$.

Sujatha *et al.* (1994) evaluated 26 ginger genotypes as intercrop in coconut plantation. Highest sprouting percentage (100 %) was recorded for cultivars Eranadan and Valluvanad. High fresh yield of rhizomes was obtained from Kurupampadi (365.5 g $plant^{-1}$) followed by Wyanad, Mananthody and Eranadan.

Among the 21 ginger genotypes studied by Chandra and Govind (1999) in Meghalaya, maximum fibre content (7.6 %) was recorded in Khasi Local and lowest in Nadia. However, Tura was high yielding (26.69 t ha^{-1}) followed by Poona (25.04 t ha^{-1}) and Basar Local (24.88 t ha^{-1}).

Singh *et al.* (1999) evaluated 18 ginger genotypes for growth and yield and reported that Thinladium, Nadia and Khasi local were tallest and had more tillers per plant. These cultivars also gave highest rhizome yield (> 30 t ha^{-1}).

Ravindran and Nirmal Babu (2005) reported that, yield and quality parameters vary with soil type, season, cultural practices, climatic conditions and genotypes.

Bala *et al.* (2007) reported higher rhizome yield of 8.16 kg $3m^{-2}$ from PLS-4 which was at par with Himgiri (7.66 kg $3m^{-2}$) among 21 ginger genotypes evaluated in Himachal Pradesh.

Jyotsna *et al.* (2012) studied four ginger genotypes under organic cultivation in Manipur. Among the genotypes Bhaise showed earliness in sprouting, produced taller plants, more number of tillers per clump, higher canopy spread, leaf area index and yield. Highest rhizome yield of 20.46 t ha^{-1} was recorded by Bhaise followed by Gorubathane (19.13 t ha^{-1}).

Chongtham *et al.* (2013) studied 10 ginger genotypes in Southern West Bengal for different yield parameters. Yield attributes like length of primary fingers varied from 3.00 to 3.53 cm and length of secondary while fingers varied from 2.53 to 3.73 cm. The diameter of primary fingers ranged from 1.71 to 2.28 cm and that of secondary fingers ranged from 1.65 to 1.95 cm. The rhizome yield per plant and projected yield were maximum in Gorubathane (201 g and 18.27 t ha^{-1} respectively). Maximum dry recovery (33.48%) was recorded with Sambuk, a local cultivar.

Jatoi and Watanabe (2013) studied 19 ginger accessions and it was revealed that high variance for plant height, rhizome weight, rhizome thickness, sheath length, tillers/plant and leaf length.

In the trial conducted by Shetty *et al.* (2015) by including 10 cultivars of ginger in the hill zone of Karnataka, cv. Maran recorded the highest plant height (71.80 cm), number of tillers per plant (23.43), number of leaves per clump (308.33), leaf area (45.06 cm^2) and leaf area index (15.41). Same cultivar

recorded higher fresh rhizome yield (29.37 t ha^{-1}) followed by Rio de Janeiro (28.04 t ha^{-1}) and Karkal local (25.84 t ha^{-1}).

Ten varieties of ginger were evaluated for different growth and yield parameters under shade net conditions by Babu *et al.* (2017). Suprabha recorded higher values in almost all the vegetative and yield attributing characters. It also recorded the highest plant height (89.83 cm) followed by Himachal (84.63 cm), Pundibari (82.56 cm) and Jalsingapara local (82.06 cm) and were on parwith each other and the lowest plant height (72.56 cm) was recorded in Maran. Number of tillers per plant (28.56) and number of leaves (245.16) was highest in Suprabha followed by Himachal (24.33; 241.83). Suprabha recorded higher number of finger rhizomes per plant (25.40), more number of primary rhizomes per plant (8.39), highest number of secondary rhizomes per plant (21.66), highest fresh rhizome yield per plant and projected yield (305 g and 32.02 t ha^{-1}).

Balakumbahan and Joshua (2017) evaluated 24 ginger genotypes under high rainfall zone of Tamil Nadu. Plant height ranged between 55.70 to 82.50 cm. The maximum plant height of 82.50 cm was observed in Pechiparai local genotype. More number of leaves per plant and highest number of tillers were observed in Sengottai local (103.2 and 12.3 respectively). Highest total leaf area of 7732.76 cm^2 was recorded in IISR Varada which was at par with Sengottai local (7668.06 cm^2). Sengottai local recorded highest fresh and dry rhizome yield per plant (227.47 g and 48.57 g respectively) and 22.16 t ha^{-1} of estimated rhizome yield. This was closely followed by IISR Varada which recorded 224.16 g $plant^{-1}$ fresh rhizome, 47.73g $plant^{-1}$ dry rhizome and 20.80 t ha^{-1} estimated rhizome yield. Dry recovery percentageof ginger genotypes ranged between 16.68 % to 22.47 %, the maximum dry recovery of 22.47 % was observed in the genotype Narasipatnam local followed by 22.33 % in Sengottai local.

Goudar *et al.* (2017a) evaluated 13 genotypes for growth and yield parameters. Among the vegetative parameters, maximum plant height was recorded in Humnabad Local (56.60 cm), which was on par with IISR Rejatha (53.25 cm), Bidar - 2 (53.20 cm) and Bidar - 1 (50.10 cm). Maximum number of leaves per clump was recorded in Humnabad Local (257.60) which was on par with Bidar - 2 (242.93) and IISR Rejatha (226.90). The stem girth varied from 0.88 cm in Suruchi to 1.21 cm in Humnabad Local. Maximum number of primary rhizome was observed in Humnabad Local (6.20) which was on par with IISR Rejatha (5.90), Bidar - 2 (5.60) and IISR Mahima (5.20). Fresh yield per plant ranged from 121.67 to 235.26 g. The highest fresh yield per plant was recorded in Humnabad Local (235.26 g) which was on par with IISR Rejatha (217.32 g), IISR Mahima (211.67 g) and Himagiri (206.45 g). Projected yield was also highest in Humnabad local (23.93 t ha^{-1}) which was at par with IISR Rejatha (21.63 t ha^{-1}). Percentage of dry ginger recovery ranged from 18.47 to 26.32. The maximum dry ginger recovery was noticed in Humnabad Local (26.32 %) which was on par with IISR Rejatha (24.95 %), IISR Mahima (24.17 %), Himagiri (22.65 %) and Suravi (22.56 %).

Karthik *et al.* (2017) evaluated 16 ginger genotypes for growth and yield in Gangetic alluvial plains of West Bengal. Among the genotypes, most promising genotype was Acc. 219 in terms of number of leaves per tiller (20.07), length of fingers (8.41 cm), girth of fingers (3.11 cm), number of fingers per clump (18.46), length of clump (20.65 cm), breadth of clump (9.88 cm), yield per plant (233.38 g) and projected yield (18.32 t ha^{-1}). Plant height (57.69 cm) and number of tillers per clump (15.87) were found maximum in Gorubathane.

Ravi *et al.,* (2017) studied 16 genotypes in Uttara Kannada district of Karnataka. The investigation indicated Humanabad Local as the most promising genotype in terms of growth and yield. The growth attributes like plant height (53.25 cm), number of leaves (315) and leaf area index (20.19) and yield attributes like length of primary fingers (7.41 cm), length of secondary fingers (5.78 cm), rhizome yield $plant^{-1}$ (360.20 g) were highest with Humanabad Local. The projected fresh yield was maximum (21.55 t ha^{-1}) and dry recovery (27.35 %) were maximum in Humanabad Local.

Among the eight ginger genotypes evaluated in Terai region of West Bengal, GCP-49 was found to be the superior genotype in terms of plant height (56.43 cm), number of leaves per plant (14.72 cm), pseudo stem girth (2.88), leaf length (21.20 cm), leaf breadth (2.54 cm), projected yield (22.55 t ha^{-1}) and dry recovery (21.7 %) (Chakraborty *et al.,* 2018).

2.1.2 Genetic variability, heritability and genetic advance

Mohanty and Sharma (1979) reported that, expected genetic advance and heritability estimates were high for number of secondary rhizome and total root weight. Genetic coefficient of variation was high for weight of root tubers.

Genetic variability parameters in 29 genotypes revealed that, phenotypic coefficient of variability (PCV) was of higher magnitude than corresponding genotypic coefficient of variability (GCV). Higher magnitude of PCV and GCV were observed for plant height, number of suckers and fingers, weight of primary rhizome and yield per plant indicating presence of wide range of variability for these traits. High heritability coupled with high Genetic Advance (GA) was observed for suckers per plant, weight of fingers and primary rhizome and yield per plant (Pandey and Dobhal, 1993).

Ali *et al.* (1994) conducted a study on genetic coefficient of variation, heritability and genetic advance for 6 traits in 16 ginger genotypes. The differences among the genotypes were highly significant for all the characters studied. The number of leaves and nodes per plant had high heritability combined with high genetic advance.

Yadav (1999) estimated coefficient of variation, heritability and genetic advance for 18 important horticultural traits in 26 accessions of ginger at Raigarh. Higher coefficient of variation was observed for length and weight of secondary rhizomes, weight of primary rhizome, number of secondary and primary rhizomes and rhizome yield per plant. High heritability coupled with high genetic advance as percentage of mean was observed for plant height, leaf length, suckers per plant, number of mother and secondary rhizomes, weight of primary rhizome and rhizome yield per plant. This indicated that desirable improvement in these traits can be brought through straight selection.

Medhi *et al.* (2007) evaluated 13 ginger genotypes for genetic variability in Port Blair. Significant differences among the genotypes along with wide range of variability were observed in all the traits. There was a close relationship between the GCV and PCV for plant height, revealing very little influence of environment for its expression. The estimate of heritability for the trait was more than 80 %.

Genetic advance in percentage of mean along with heritability was very high for tillers per plant, plant height, leaf length, leaf breadth, leaves per tiller, number of primary fingers per rhizome, number of secondary fingers per rhizome and number of tertiary fingers per rhizome among nineteen ginger genotypes. Phenotypic Coefficient of Variation was more or less same or little bit higher than Genotypic Coefficient of Variation for all these characters (Islam *et al.,* 2008).

Genetic variability among 36 accessions of ginger was studied by Aragaw *et al.* (2011) at two locations in Ethiopia. Results showed that fresh rhizome yield and dry rhizome yield showed high GCA and PCV at both locations. Relatively high heritability and genetic advance was obtained for oleoresin, volatile oil, fibre content, fresh and dry rhizome yield. Wide variability was observed among the genotypes.

Parmar (2011) studied genetic variability in 30 ginger genotypes in Himachal Pradesh. High GCV, heritability and genetic advance were found in plant height, number of leaves per plant and dry weight of rhizomes.

Five ginger varieties were evaluated by Lakshmi and Rajasekhar (2013), high heritability values were recorded for plant height, number of tillers per plant, number of mother and finger rhizomes per plant and fresh rhizome yield per plant. Genetic advance in percentage of mean along with heritability was very high for fresh rhizome yield per plant, number of mother rhizomes per plant and number of tillers per plant. Phenotypic coefficient of variation was higher than genotypic coefficient of variation for all these characters.

Twenty five ginger accessions collected from different locations tested for 13 diverse traits. High GCV was found for acidity, oleoresin content, ascorbic acid, yield per plant and TSS. Based on genetic variability analysis, only six genotypes out of 25 genotypes *viz.* Sultanpur-2, FZD- 2, NDG-41, NDG-8, NDG-22 and NDG-18 were found to be most promising for rhizome yield and quality traits. The influence of environment was expected to be minimum when difference between GCV and PCV was less in magnitude for all studied characters. Based on high heritability coefficient (h^2bs) along with high genetic advance as per cent mean, oleoresin content (0.98, 76.36%), ascorbic acid content (0.97, 70.42), acidity % (0.93, 85.45%), TSS per

cent(0.90, 43.71) and yield per plant (0.87, 45.69) were found superior traits representing additive genetic variance (Ravishanker *et al.,* 2013).

Islam *et al.* (2017) studied 20 ginger genotypes for nine morphological traits. Maximum variation was obtained for disease infection (%) and yield (t/ha) followed by yield per plant (g) and weight of secondary rhizome per hill (g).

2.1.3 Correlation and path coefficient analysis

In a study by Saikia and Shadeque (1992) parameters such as leaves per clump, tillers per clump and shoot height showed positive correlation with yield.

Sasikumar *et al.* (1992) reported that, in the evaluation of 100 genotypes for different morphological and yield characters, positive significant association with rhizome yield was observed for plant height, leaf number, tiller number as well as length and width of leaves. Plant height followed by leaf length showed maximum direct effect on rhizome yield.

Pandey and Dobhal (1993) reported that rhizome yield per plant was positively associated with plant height, number of fingers per plant, weight of fingers and weight of primary rhizome among 29 ginger collections. Path analysis revealed that weight of fingers, width of fingers and leaf width were the strongest forces influencing yield.

Chandra and Govind (1999) studied 21 ginger genotypes for three consecutive years. Number of leaves per clump, weight of mother rhizome and internodal distance of rhizome were found to be the most variable characters (21.3-32.9% CV). Yield was positively and significantly correlated with tillers/clump (r=0.83), internodal distance of rhizome (r=0.51 and plant height (r=0.50) and was negatively correlated with fibre content (r=0.53).

Among the 40 genotypes, the correlation coefficient showed the positive association of yield with leaflet number (0.6803) followed by height (0.62188), rhizome length (0.6135), leaf length (0.5824) and thickness of secondary rhizome (0.5172). Path analysis revealed that characters such as leaflet number, rhizome length, thickness of secondary rhizome, rhizome width and leaflet length have high positive direct effect with yield. Leaflet number showed high significant association coupled· with high positive direct effect, which indicates the importance of this character in selection for yield improvement (Abraham and Latha, 2003).

Bala *et al.* (2007) reported that, yield per plot was positively correlated with essential oil while negatively correlated with dry matter, oleoresin and crude fibre.

Jatoi and Watanabe (2013) studied 19 ginger accessions. Positive and significant correlation among different quantitative traits was observed. Plant height, leaves/tillers and tiller thickness appeared to be of prime importance as they directly influence rhizome yield.

Lakshmi and Rajasekhar (2013) reported that, in the evaluation of five ginger genotypes all the vegetative and rhizome characters showed positive correlation with rhizome yield. But, number of mother and finger rhizomes per plant showed the highest positive and significant correlation with rhizome yield per plant.

In 25 genotypes, genotypic correlation coefficient revealed that, rhizome yield had significant positive correlation with length of primary finger (0.40), ascorbic acid content (0.37), plant height (0.36), number of primary fingers (0.35) and diameter of primary finger (0.31) (Ravishanker *et al.,* 2013).

2.1.4 Genetic diversity

Singh *et al.* (2000) carried out D^2 genetic diversity analysis showed that 18 genotypes were grouped into three major clusters. Cluster I consisted of 16 genotypes of which nine were from Meghalaya, two each from Assam and Kerala and one each from Brazil, Maharashtra and Himachal Pradesh. Cluster II and III had

only one genotype from Meghalaya and Assam respectively. The major factors for divergence were rhizome yield per plant, oleoresin and fibre content.

In a study conducted by Aragaw *et al.* (2011) at two locations *i.e.,* Tepi and Bahir Dar of Ethiopia, D^2 analysis showed that, 36 genotypes were grouped into seven clusters in the study at Tepi and 11 clusters in the study at Bahir Dar. This makes the genotypes to become highly divergent. The overall assessment showed that, there is wide variability among ginger accessions in Ethiopia which is an important implication for breeding ginger for yield, morphological and yield attributes.

Parmar (2011) studied genetic variability of 30 ginger genotypes, D^2 analysis showed that, 30 genotypes grouped into five clusters. Clustering of genotypes from different places of collection indicated that, genetic divergence is not related to place of collection. Clustering of genotypes from different places of collection within a cluster could be attributed to the possibility of free exchange of breeding materials.

Islam *et al.* (2017) studied the genetic diversity in 20 ginger genotypes. Genotypes were grouped into five different clusters. Maximum number of genotypes (6) was grouped in cluster IV and V followed by cluster II (4 genotypes). Intra-group distance was lesser than the inter group distance. It suggested wider genetic diversity of the lines among the clusters. The intra-cluster distances in all the clusters were found low indicating that the genotypes within the clusters are closely related.

2.2 Molecular characterization

DNA markers are fragment of DNA revealing mutations/variations, which can be used to detect polymorphism between different genotypes or alleles of a gene for a particular sequence of DNA in a population or gene pool. Such fragments are associated with a certain location within the genome and may be detected by means of certain molecular technology. Among the different techniques that are extensively used are the restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), microsatellites or simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) (Jiang, 2013).

In ginger molecular characterization is reported by using AFLP, RAPD, ISSR, micro satellite (SSR) and SNP markers.

2.2.1 Molecular characterization by Random amplified polymorphic DNA (RAPD)

Random amplified polymorphic DNA (RAPD) is an easy, cost-effective, less time consuming molecular marker and employed quite often for the study of genetic diversity. The RAPD marker system can be used to detect inter-specific gene flow, analyze hybrid speciation and determine taxonomic identity (Hadrys *et al*., 1992). It is also useful in distinguishing individuals, cultivars or accessions (Karp *et al*., 1996) and identification of asexually reproduced plant varieties for forensic or agricultural purposes (Congiu *et al*., 2000).

The RAPD analysis revealed a differential polymorphism of DNA, where polymorphic bands ranged from 26 to 70 among 16 cultivars studied. The RAPD primers OPC02, OPA02, OPD20 and OPN06 showed strong resolving power and were able to distinguish all 16 cultivars. Relatively high genetic variation was detected among the cultivars (Nayak *et al.,* 2005).

Genetic diversity of 20 Chinese ginger cultivars using RAPD primers has been reported by Zhenwei *et al*. (2006). Twenty five RAPD primers produced a total of 171 DNA amplicons with size range from 100-3500 bp, out of which 111 were polymorphic. Based on the RAPD data phylogenetic tree was constructed to unravel the relationship among cultivars.

Palai and Rout (2007) studied genetic variation in eight ginger genotypes using RAPD markers. By using 12 primers, 55 distinct DNA fragments ranging from 0.5-2.4 Kb were amplified. In the cluster analysis, two major clusters were formed. First major cluster had only one genotype S-558 with 43% similarity with other seven genotypes. Second major cluster was sub divided into two minor clusters with six genotypes (Jugijan, Turialocal, Nadia, Zo-17, Nahfrey and Gurubathan) in one and another with Surabhi.

Molecular fingerprinting was done by using RAPD and ISSR markers in seven ginger genotypes which included elite, exotic and primitive genotypes. In RAPD markers, maximum polymorphism (40%) was observed in the case of primer OPJ05 and OPC11. Out of 16 primers, six primers produced unique bands. Three primers *viz.* OPB-19, OPC-13, OPE-11 generated discrete bands in Pink ginger. OPJ-07 produced a unique band in IISR Varada. While the primers OPA-08 and OPB-05 generated bands specific to Kintoki and Kozhikkalan respectively. Dendrogram showed single cluster with four groups. First group was having Pink ginger and Kintoki was grouped in the second group. Primitive ginger types *viz.,* Ellakkalan, Kakkakalan, Kozhikalan and Sabarimala formed third cluster and they showed maximum similarity (97%) between them. The released variety IISR Varada formed fourth group. The exotic Kintoki had high similarity (80%) with indigenous primitive type Pink ginger and with putative wild forms (88%). IISR Varada showed high similarity with primitive types (92 %-93 %) followed by Pink ginger (86%) and Kintoki (85%). Findings from this study imply that, these putative types may be progenitors of elite cultivars (Prem *et al.,* 2008).

Jaleel and Sasikumar (2010) studied the genetic diversity in 46 accessions of ginger using RAPD and ISSR markers. Sixty RAPD and 17 ISSR primers were used. In RAPD, 269 scorable bands were produced and out of which 126 were polymorphic. In case of ISSR, 76 bands were polymorphic out of 160 scorable bands. In the dendrogram, improved varieties/cultivars were grouped together with primitive types. It was also found that, in the clustering pattern a geographical bias was also evident. Most of the improved varieties grouped distinctly from the land races/cultivars in all the clusters. Primitive type gingers such as Sabarimala (Acc. 246), Kozhikkalan (Acc. 537), Ellakallan (Acc. 463) *etc*. are grouped in the first cluster and showed close similarity with the landraces, Acc. 27, Acc. 20 and Acc. 295 as well as the improved varieties, IISR Varada (Acc. 64), IISR Mahima (Acc. 117), and IISR Rejatha (Acc. 35) indicating that the primitive types may be the progenitor of the present day ginger varieties. Accessions collected from same geographical area clustered together. The accessions that stood singly in the dendrograms were rather unique entities in one or other aspects. Three primitive gingers, Pink ginger (Acc. 731), Kintoki (Acc. 648) and Kakakalan (Acc. 558) clustered singly in all the three dendrograms.

Sajeev e*t al.* (2011) carried out genetic diversity analysis in 49 ginger clones from North-East India using 30 random amplified polymorphic DNA (RAPD) markers. Out of 30 primers, 18 showed reproducible polymorphic bands. Five clusters were identified by Jaccard's genetic similarity. Principal component analysis of the molecular data supported grouping of the clones into six hypothetical populations based on their source or location of collection.

Twelve ginger accessions were assessed for diversity analysis using RAPD markers. Thirteen out of 20 primers screened were informative and produced 275 amplification products, among which 261 products (94.90%) were found to be polymorphic. The percentage of polymorphism varied from 88.23% to 100%. Maximum number of bands was produced by OPA-11(28). Dendrogram showed that, the genotypes were grouped according to place of collection of the genotypes (Ashraf *et al.,* 2014).

2.2.2 Molecular characterization by Simple Sequence Repeats (SSRs)

Simple Sequence Repeats (SSRs) or microsatellites, which are genetic markers that identify alleles with high repeatability and reproducibility (Gutierrez *et al*., 2005), are widely used in taxonomy and diversity studies. SSRs appear to be ubiquitous in higher organisms, although their frequency varies among species. They are generally abundant, dispersed throughout the genome and also show higher levels of polymorphism than other genetic markers. Furthermore, they are co dominantly inherited and suitable for automation, which has additional advantages over other types of molecular markers (Holton, 2001). The sequences flanking specific microsatellite loci in a genome are considered to be conserved within species, across species in a genus and perhaps even across related genera (Varshney *et al.,* 2002). Often, microsatellites developed in one species have been used in a different species, demonstrating their transferability and ability to detect polymorphism.

Jatoi *et al.* (2006) used rice microsatellite markers to assess the genetic variability among the three genera of Zingiberaceae family: Zingiber, Alpinia and Curcuma. The origin of the genotypes was diverse, covering eight Asian countries. Among the 141 bands, 140 (99.5%) were polymorphic. On an average, each microsatellite primer set amplified 17.6 DNA fragments.

Lee *et al.* (2007) characterized 10 accessions of ginger using 154 primer pairs. Only eight primer pairs produced reproducible polymorphic bands and were further characterized using 20 accessions. The eight polymorphic loci revealed a total of 34 alleles across the 20 accessions, ranging from two to seven alleles with an average of 4.3 alleles per locus. The values for observed and expected heterozygosity ranged from 0 to 1.0 and from 0.23 to 0.67, respectively. The heterozygote deficits were observed at three loci (GB-ZOM-040, GB-ZOM-064 and GB-ZOM-111).

Three Malaysian ginger cultivars (Bukit Tinggi, Tanjung Sepat and Sabah) were examined for genetic polymorphisms using single microsatellite oligonucleotide primers (CATA)5, (GATA)5 and (GAC)6 microsatellite DNA primers. Seven polymorphic bands with 2.334 polymorphic bands per primer were obtained. Cluster analysis revealed 87.50% similarity between Bukit Tinggi and Tanjung Sepat, 64.27% similarity between Bukit Tinggi and Sabah and 56.25% similarity between Tanjung Sepat and Sabah (Mahdi *et al.,* 2013).

Eighteen ginger cultivars from Northwest Himalayan region were characterized both by chemical (through HPLC) and genetic analysis (ISSR and SSR markers). Moderate to high diversity was observed in the collections. SSR markers were observed to be better in displaying average polymorphism (77.8%) than ISSR (66.7%). One ISSR and two SSR primers could be identified which effectively distinguished closely related ginger cultivars. Chemical profiling and subsequent multivariate analysis distinguished five lines which were distinct from rest of the collection. However, the present study could not correlate chemical profile with molecular marker (Pandotra *et al.,* 2013).

Das *et al.* (2016) studied genetic diversity in 60 ginger genotypes using molecular markers. Thirteen SSR markers were used and 160 polymorphic bands were observed. Variation among different ginger population was 66 % and variation found within the population was 34 %. These results showed that, ginger cultivars display significant genetic diversity at the population level. Dendrogram revealed that, grouping of accessions indicated that accessions were grouped in relation to area of collection, indicated geographical closeness due to genetic similarity irrespective of the relation that exists at the morphological level.

2.2.3 Single Nucleotide Polymorphism (SNPs)

Single nucleotide polymorphisms (SNPs) are the polymorphism occurring between DNA samples with respect to single base. SNPs comprise most abundant molecular markers in the genome. SNPs have become the markers of choice. Due to their abundance in genome, they are extremely useful for creating high-density genetic map. SNPs have the potential to provide basis of a superior and highly informative genotyping assay. SNPs in coding region may have functional significance if the resulting amino acid change causes the altered phenotype. SNP markers associated with phenotypic changes pinpoint functional polymorphism. They seem to comprise the largest class of functional polymorphisms (Jehan and Lakhanpaul, 2006).

SNPs are less mutable as compared to other markers, particularly microsatellites. The low rates of recurrent mutations make them evolutionarily stable. They are excellent markers for studying complex genetic traits and for understanding the genomic evolution. This also makes them suitable and easier to follow in population studies (Jehan and Lakhanpaul, 2006).

Among all molecular markers, SNPs are the most abundant and powerful, feasible for automated high-throughput genotyping and available for multiple assay options using different technology platforms to meet the demand for genetic studies and molecular breeding in crop plants (Steemers and Gunderson 2007; Alkan *et al*., 2011). In present time, SNPs have gained much interest in the scientific and breeding community, that could be used as potential DNA markers, which may be identified practically in every gene (Rafalski, 2002). SNPs can also identify the genomic diversity of species to demonstrate the speciation and evolution and associate genomic variations with phenotypic traits (McNally *et al*., 2009).

Studies on application of SNPs in identification of ginger diversity are almost nil. SNPs, which are used as markers for genetic studies, can be detected using expressed sequence tags (EST). Reviews on identification of SNPs in other crops are also given below.

Chandrasekar *et al.* (2009) mined 38139 ginger EST sequences from dbEST of NCBI. 64026 SNP sites and 7034 indel polymorphisms with frequency of 0.84 SNPs / 100 bp found in ginger. Among three

tissue from which EST libraries generated, rhizomes had high frequency of 1.08 SNPs/indels per 100 bp followed by roots which had 0.82/100 bp and lowest was in leaf which was 0.63/100 bp. In the detected SNPs, transversion was high compared to transition. It is reported from this study that, transversion was high because ginger is vegetatively propagated through rhizomes.

Hamilton *et al.* (2011) discovered 575,340 SNPs by sequencing normalized cDNA prepared from three commercial potato cultivars (Atlantic, Premier Russet, and Snowden).

Hamilton *et al.* (2012) identifies SNPs in tomato by whole transcriptome sequencing of six accessions that span cultivated market classes. A total of 62,576 non-redundant putative SNPs were identified. The SNPs within the contigs were present within all of the GO molecular function categories. The computational pipeline had validation rates in SNP genotyping assays that ranged from 95 to 100% and the utility of these SNPs for assessing genetic variation within cultivated and wild populations was demonstrated. Collectively, the transcript sequences and the annotated SNPs provide a resource to facilitate tomato genetics and breeding efforts.

Kim *et al.* (2014) discovered 4,680,647 putative SNPs from two accessions of *Solanum pimpinellifolium* by comparing with reference, of which 89.9% (4,210,454) were homo and 10.1% (470,193) were hetero-type SNPs. The total number of SNP and the density of SNP in different chromosomes also varied widely. An average 6.1 SNPs/kb was observed in the whole genome.

Transcriptome of *Phoebe chekiangensis* was sequenced using next-generation sequencing technology and 75,647 transcripts with 48,011 unigenes were assembled and annotated. In addition, 162,938 putative single nucleotide polymorphisms (SNPs) were predicted and 25 were further validated (He *et al.,* 2017).

2.3 Biochemical characterization

2.3.1 Oleoresin

Oleoresin represents the cumulative effect of the sensation of smell and taste of ginger. It consists of the volatile essential oil and the non volatile resinous fraction comprising taste of components, fixatives, antioxidants, pigment and fixed oils naturally present in the spice (Govindarajan, 1982). The oleoresin yield of ginger varies from 3 to 11%. It depends on the solvent extraction conditions, the state of rhizomes (fresh or dried), place of origin and harvest season (Vernin and Parkanyi, 2005) in addition to genotype. Besides organic solvent extraction, liquid carbon dioxide is used for the extraction of oleoresin from ginger (Sankamura and Hayashi, 1978). Nobrega *et al*., (1997) compared the ginger oleoresin obtained with ethanol, isopropanol and pressurized CO_2. The pungent compounds were detected in oleoresin obtained with organic solvent (extraction time of 6 hours) and in samples obtained with CO_2 liquid (extraction time of two hours).

Ratnambal *et al.* (1987) reported that, the percentage of essential oil and oleoresin content decreased with increase in maturity in ginger. Among the 100 genotypes studied oleoresin content ranged from 4.6% (Himachal) to 7.5% (PGS-37) (Sasikumar *et al.* 1992). Among the 21 ginger genotypes studied by Bala *et al.* (2007), SG-705 (5.22 %), SG-1133 (5.00 %) and SG-857 (4.58 %) recorded higher oleoresin content.

Salve and Kajalwad (2011) reported variation in oleoresin content in the fresh and dried rhizomes. Percentage of oleoresin from fresh and dried rhizome was 8.3 and 6.7% respectively from a local ginger cultivar.

Oleoresin content of 5.12 % was recorded in Bhaise in an organic trial at Manipur by Jyotsna *et al.* (2012)

Chongtham *et al.* (2013) reported highest oleoresin content of 10.25 % from ginger variety Suravi among the 10 genotypes evaluated in the sub-tropical humid region of West Bengal.

Among the 24 ginger genotypes, PGS - 24 recorded highest oleoresin content of 11.20 % followed by the genotype Sengottai local (10.90 %) (Balakumbahan and Joshua, 2017).

In a study by Goudar *et al.* (2017b) oleoresin content in twelve ginger genotypes under study varied from 3.69 to 7.35 %. Humnabad Local recorded maximum oleoresin content (7.35 %) and it was at *par* with Rejatha (6.12 %).

Oleoresin content was highest *i.e.* 4.1 % in GCP-49 among the eight ginger genotypes studied in Terai region of West Bengal (Chakraborty *et al.,* 2018).

2.3.2 Crude fibre

Quality of ginger is dependent upon the fibre content of fresh mature and dried material. In fresh mature sample, it ranges from 2-3%, while in dried ginger it is 5-6%. The immature fresh ginger will be free of fibre. Fibre content is most important factor on which the quality of commercial ginger is based on in domestic as well as international market (Arya, 2001). The fibre, volatile oil contents and the pungency level are the most important criteria in assessing the suitability of ginger rhizomes for particular processing purposes.Young, tender rhizomes lifted at the beginning of the harvesting season, about 5–7 months after planting, are preferred for the manufacture of preserved ginger since the fibre content is negligible and the pungency is mild. As the season progresses, the relative abundance (on a dry weight basis) of the volatile oil, the pungent constituents and the fibre increases. At about nine months after planting, the volatile oil and pungent principle contents reach a maximum and thereafter their relative abundance falls as the fibre content continues to increase (Zachariah, 2008).

Fiber content is one of the most important criteria for assessing the suitability of ginger rhizome for its value addition like ginger paste, salted ginger, ginger powder *etc.* (Jaleel and Sasikumar, 2009).

Crude fiber content of dried ginger usually ranges from 4.8 to 9.0% (Natarajan *et al*., 1972). Among the 21 ginger genotypes studied by Bala *et al.* (2007), SG-1046 (2.11 %), and SG-1021 (3.45 %) recorded minimum fibre content. Ugwoke and Nzekwe (2010) reported 0.92% crude fibre content from a Nigerian ginger cultivar. Crude fibre content of 5.71% was recorded in Bhaise in an organic trial at Manipur by Jyotsna *et al.* (2012).

Fiber content estimated in the 24 genotypes by Balakumbahan and Joshua (2017), revealed that, highest fiber content of 9.56% was recorded in Suprabha followed by 7.41% in V_1S_1 - 8. The best fresh rhizome yielder Sengottai local recorded 5.42% of fiber content.

In a study by Goudar *et al.* (2017b) crude fibre content in 12 genotypes varied from 3.34 to 5.72 %. Minimum fibre content was recorded in Varada (3.34 %), which was on par with Rejatha (3.42 %), Suravi (3.62 %), Jorhat-2 (3.72 %) and Humnabad Local (3.86 %).

2.3.3 Essential oil

Essential oil in ginger ranges from 0.2 to 3 %, based on the origin of genotype and maturity stage of the rhizome (Ekundayo *et al.,* 1988 and Van *et al*., 1987). In ginger, major part of the essential oil is located in the epidermal part of the rhizome (Mangalakumari *et al*., 1984). Ginger owes its unique flavour properties to the combination of pungency and aroma. The pungency is provided by non-volatile phenolic compounds, whereas, the essential oil gives ginger its characteristic aroma. Since the nature of the composition of the volatile oils influences the aroma and flavour of ginger, these quality parameters are indispensable in determining the various grades and price of rhizomes. Ginger oil obtained by supercritical fluid extraction using carbon dioxide differs radically from steam distilled oil due to the presence of the pungent gingerols and shogoals (Bartley and Jacobs, 2000). Steam distillation of dried rhizomes of ginger yields 0.2 to 3.0 % essential oil (Krishnamurthy *et al.,* 1972; Connel and Jordan, 1971; Anzaldo *et al.,* 1986 and Ekundayo *et al*., 1988). The volatile oil components were mainly mono and sesquiterpenes; camphene, beta-phellandrene, curcumene, cineole, geranyl acetate, terphineol, terpenes, bomeol, geraniol, limonene, linalool, alpha-zingiberene (30-70 %), beta-sesquiphellandrene (15-20 %), beta-bisabolene (10-15 %) and alpha-farmesene (Vernin and Parkanyi, 2005).

A number of sesquiterpenes *viz.,* alpha copaene, beta bourborene, alpha bergamotene, alpha selinene chalamenene and cuparene which were not reported earlier in ginger oil were identified from essential oils of ginger from Fiji (Smith and Robinson, 1981). Salzer (1975) suggested that, ar-curcumene might be originally absent from ginger and be formed on storage by the decomposition of zingiberene and beta sesqiphellandrene. A similar shift from zingiberene to ar-curcumene was also observed by Connel and Jordan (1971). But, Ekundayo *et al.* (1988) found that ar-curcumene is one of the major components in the essential oil isolated from fresh and dried ginger.

Zingiberene, beta phellandrene, alpha-Pinene, camphene, geranial, neral and 1,8-cineole appear to be a typical constituents of ginger oils (Gopalam and Ratnambal, 1989).

Gas chromatographic pattern of essential oil is related to its source of origin. Variability in peak area has been observed in Jamaican, African and Cochin ginger oils (Connel and Jordan, 1971). Literature shows varying concentration of zingiberene in the rhizome oils of plant growing in India (46.2 %) (Vernin and Parkanyi, 1994), China (38.12 %) (Vernin and Parkanyi, 1994), Nigeria (29 %), Australia (20-28 %) (Connel and Jordan, 1971), Mauritius (9.5%) (Fakim *et al*., 2002), the Central African Republic (3.6%) (Menut *et al*., 1994) and Sri Lanka (1.3%) (Macleod and Pieris, 1984).

Chemical composition of volatile oils of ginger from Nigerian origin was investigated by means of GC and GC-MS. The essential oil contained mainly geranial, neral, 1, 8- cineole, zingiberene, beta bisabolene and beta sesquiphellandrene. Geranial was found to be the most abundant component in ginger oil distilled from fresh ginger rhizome. The volatile components were predominantly mono and sesquiterpinoids. The major mono terpenes identified in ginger essential oils are neral, geranial, camphene and 1, 8-cineole, whereas the major sesquiterpenoids are zingiberene, beta sesquiphellandrene, beta bisabolene and ar-curcumene. Generally there is a reduction in the monoterpenoid contents and increase in the amount of sesquiterpenes hydrocarbon on drying the rhizome (Ekundayo *et al.,* 1988).

Miyazawa and Kameoka (1988) identified 72 components in the volatile oil extracted from the air-dried rhizomes. The main components were α-zingiberene (21.8%), geranial (9.9%), geraniol (9.4%), β-bisabolene (7.9%), nerol (7.1%), 1,8-cineol (6.2%), α-terpineol (5.6%), borneol (5.4%), β-phellandrene (3.1%), linalool (1.7%), methyl nonyl ketone (1.6%) and camphene (1.4%); the other components accounted for ∼ 1% each of the volatile oil.

Twenty ginger varieties evaluated for quality attributes in Assam by Saikia and Shadeque (1992), among these varieties highest crude fibre (8.05%) was reported in Moran followed by Jorhaut hard (7.86%). These varieties also recorded highest volatile oil (2.85 and 2.56% respectively).

Zachariah and Sasikumar (1998) evaluated 41 ginger accessions for oil, oleoresin, gingerols and shogaols. Wide variability was noticed in the germplasm for these quality characters. Accessions with high oleoresin, gingerol and shogaol were Acc. 2, 22, 42, 60, 86, 141 and 197.

Yu *et al*. (1998) reported the presence of monoterpenes and sesquiterpenes as the main components in three samples of steam distilled oil. They could not find pungent components in oil. Besides sesquiterpenes, the supercritical CO_2-extracted ginger oils contained 18.61–23.09 % pungent components. These oils preserve the typical spicy odour and pungency of ginger.

The composition of the essential oil hydrodistilled from dried Nigerian ginger was determined by GC and GC-MS techniques. The oil yield was 2.4 % and the oil consisted of 64.4 % sesquiterpene hydrocarbons, 6.6 % carbonyl compounds, 5.6 % alcohols, 2.4 % monoterpene hydrocarbons and 1.6 % esters. The main compounds were zingiberene (29.5 %) and sesquiphellandrene (18.4 %). A number of constituents not previously reported in ginger oil were identified. These included 2,6-dimethyl hepten-l-ol, α-gurjunene, linalool oxide, isovaleraldehyde, 2-pentanone, cadinol, α- and γ-calacorene, eremophyllene, Tmuurolol, α-himachallene, α-cubebene acetic acid, pinanol, α-santalene, geranyl propionate, geranoic acid, (E,E)-α-farnesene, n-methyl pyrrole and geranic acid (Onyenekwe and Hashimoto, 1999).

Sultan *et al.* (2005) evaluated quality of ginger rhizomes from China and Thailand. Essential oil content was 0.98% in China sample and 1.58% in Thailand sample which was approximately 60% higher essential oil content compared to China sample. By GC analysis of essential oil it was found that, Thailand

sample contains higher zingibrene (30.81%) compared to China sample (8.0%). Concentration of zingebrene was 70% higher.

Wohlmuth *et al.* (2006) studied the essential oil composition in 17 clones of Australian ginger, including commercial cultivars and tetraploid clones using GC/MS. The oil was mainly composed of high level of citral and relatively low level of sesquiterpene hydrocarbons. But, one clone, the cultivar Jamaican, yielded oil with a substantially different composition, lower citral content and higher level of sesquiterpene hydrocarbons.

Among the 21 ginger genotypes studied by Bala *et al.* (2007), SG-810 (1.32 %), and PLS-4 (1.31 %) recorded higher essential oil content.

Menon (2007) carried out GC/MS analysis of essential oil from fresh leaves and fibrous roots of two local cultivars of ginger *viz*. Kozhikkalan and Vellinchi. Oil was extracted from fresh as well as dried ginger by hydro distillation. Geranial and zingerone were the major compounds present in fresh ginger and their content decreased during processing. The contents of ar-curcumene, zingiberene, beta-bisabolene, germacrene-D, beta sesquiphellandrene, gamacadinene, beta famesene, beta elemene, alpha bergamotene and gamacadinene were increased with hydro distillation.

Toure and Xiaoming (2007) reported 0.44% and 0.22% essential oil on fresh weight basis in Guinean and Chinese ginger respectively. GC-MS analysis of oil showed that zinziberene was the most abundant compound identified for Guinean and Chinese ginger (19.98 and 31.1% respectively).

The ginger rhizome contains a little steam volatile oil, fixed (fatty) oil, pungent compounds, resin, proteins, cellulose, pentosans, starch and mineral elements. Out of these compounds, starch is the most abundant which accounts for 40–60% of the rhizome on a dry weight basis. Relative abundance of certain constituents varies between samples of fresh and dried ginger. The composition of the fresh rhizome is determined by the cultivar grown, the environmental conditions of growth and the stage of maturity at harvest. Further changes in the relative abundance of some constituents can also occur postharvest during the preparation and subsequent storage of dried ginger (Zachariah, 2008).

Characterization of 46 ginger accessions revealed that, the primitive type gingers such as Sabarimala, Kozhikkalan and Kakakalan as well as few landraces have higher levels of oleoresin and essential oil compared with the improved varieties (Jaleel and Sasikumar, 2009).

Jaleel and Sasikumar (2012) profiled 46 ginger accessions for its volatile oil constituents using GC/MS. Sixty compounds constituting 71.15 to 94.57% of the total volatile oil constituents were identified. The highest percentage of essential oil constituents were identified in Acc. 50 while the lowest was identified in the exotic ginger Kintoki. Zingiberene was the major component present in the essential oils of all the 46 ginger accessions except the exotic ginger Kintoki, in which α curcumene was the major component. Highest percentage of zingiberene was observed in the cultivar, Angamali (29.6%) and the lowest percentage in Kintoki (6.79%). Z-citral (neral) and E-citral (geranial) were the important monoterpene aldehydes found in all the ginger genotypes except the exotic ginger ‘Brazil’. One of the major sesquiterpenes, farnasene, was absent in the essential oil of Acc. 50 and present in all other accessions studied. The highest percentage of farnasene was observed in the exotic ginger Brazil (15.1%) and the lowest percentage in the cultivar Ambalawayalan (4.1%). High amount of α curcumene and β sesquiphellandrene were detected in all the ginger accessions. Alpha curcumene ranged from 1.9 (Angamali and Naval parasi) to 6.66% (Suruchi) and β sesquiphellandrene ranged from 6.39 (Rio-de-Janeiro) to 12.17% (Brazil). 1, 8-cineole was found in all the ginger genotypes except (Kintoki). The content ranged from 1.08 (Bhaise) to 6.96% (Jolpaiguri). Alpha pinene, another important monoterpene observed in all the accessions, ranged from 0.46 (Mananthodi) to 1.9% (Kintoki). Beta phellandrene was absent in both (Kakakkalan) and (Jolpaiguri). Among the released varieties studied, the percentage of zingiberene was highest in Suprabha (24.56%) followed by IISR Mahima (23.95%), Suruchi (22.96%), IISR Varada (21.64%) and IISR Rejatha (20.93%). Other major compounds identified were z-citral, citral, farnesene, beta-sesquiphellandrene, α- curcumene, camphene, beta phellandrene, 1, 8-cineol, endo borenol and nerolidol. Among the primitive type gingers, the highest content of zingiberene (25.86%) and lowest content of farnesene (4.29%) were observed in the collection, Sabarimala. Citral content was high in Kakkakkalan when compared to the other primitive type gingers. The amount of β-

sesquiphellandrene was found to be almost equal among the primitive type gingers, Sabarimala, Kozhikkalan, Kakakkallan and Ellakkallan.

Kiran *et al.* (2013) chemoprofiled 17 cultivars of fresh ginger from North-East India by GC-MS analysis. Among the tested cultivars Assam Fibreless yielded highest essential oil (4.17%) on fresh weight basis. Assam Tinsukia had the highest citral content (23.66±1.60%) and Mahima had the highest zingiberene content (29.89±0.42%). Major volatile constituents including camphene (8.49±0.41%), neral (4.95±0.34%), geranial (12.36±0.46%), zingiberene (20.98±2.34%) and β-sesquiphellandrene (7.96±0.66%) were observed.

In a study by Goudar *et al.* (2017b), essential oil content in 12 ginger genotypes under study varied from 1.32 to 2.25 %. The highest essential oil content on fresh weight basis was obtained in Suravi (2.25 %) which was on par with Humnabad Local (2.13 %), IISR Rejatha (2.04 %) and Suruchi (1.93 %).

Essential oil content was highest *i.e.* 1.3 % in GCP-49 among the eight ginger genotypes studied in Terai region of West Bengal (Chakraborty *et al.,* 2018).

III. MATERIAL AND METHODS

The present investigation entitled “Molecular and chemo profiling of ginger (*Zingiber officinale* Rosc.) genotypes” was carried out during 2016-17 and 2017-18 at ICAR-Indian Institute of Spices Research, Kozhikode, Kerala and ICAR-Indian Institute of Spices Research Experimental Farm, Peruvannamuzhi, Kerala. The details of material used and methods followed during the course of investigation are presented here under.

3.1 Geographical location of the experimental site

The experimental site is located at 11°36’34” North latitude and 75°49’12” Eastlongitude.

3.2 Climatic conditions of the experimental site

The meteorological data recorded during the experimental period (June to February) are presented in Appendix-I. During the experimental period of 2016-17 maximum temperature ranged from 29.4 °C (June 2016) to 35.5 °C (February 2017), while, the minimum temperature ranged between 20.09 °C (January 2017) to 24.47 °C (August 2016). Similarly, during the course of investigation in 2016-17 maximum relative humidity of 95.61 per cent was recorded during July 2016 and lowest (88.17 %) in February. So also, the rainfall of 20.00 mm and 930.6 mm was received during December 2016 and July 2016, respectively. The maximum sunshine hours of 4.9 h was recorded during February 2017, while it was lowest (0.86 h) during July 2016.

During 2017-18, maximum temperature ranged from 29.28 °C (June 2017) to 36.34 °C (Feb. 2018), while, the minimum temperature ranged between 19.56 °C (Jan. 2018) to 24.66 °C (June 2017). Similarly, during the course of investigation in 2017-18 maximum relative humidity of 95.75 per cent was recorded during August 2017 and lowest (88.28 %) in February 2018. There was no rainfall during January and February 2018. Maximum rainfall received during the period was 837.6 mm (August 2017). The maximum sunshine hours (7.0 h) was recorded during February 2018, while it was lowest (0.81 h) during June 2017.

3.3 Varieties and genotypes

Twenty seven ginger genotypes, one *Zingiber* sp., one *Curcuma* sp. and one *Kaempferia* sp. were used in the study (Table 1). For statistical analysis only 28 genotypes were considered, whereas, for GC/MS studies and molecular characterization all the 30 genotypes were used.

Table 1: Ginger genotypes and other species used in the study with their source of collection

Sl. No.	Genotypes	Description
Released varieties		
1.	IISR Varada	Clonal selection, Released from ICAR-IISR, Kozhikode
2.	IISR Mahima	Clonal selection, Released from ICAR-IISR, Kozhikode
3.	IISR Rejatha	Clonal selection, Released from ICAR-IISR, Kozhikode
4.	Suprabha	Clonal selection, Released from HARS, OUAT, Pottangi
5.	Suravi	Induced mutant of Rudrapur local, Released from HARS, OUAT, Pottangi, Odisha
6.	Suruchi	Released from HARS, OUAT, Pottangi, Odisha
7.	Sourabh	Mutant (V_1S_1 2) from HARS, OUAT, Pottangi, Odisha
8.	Athira	Selection form somaclones of cultivar Maran, Released from KAU,Thrissur, Kerala
9.	Karthika	Selection form somaclones of cultivar Maran, Released from KAU,Thrissur, Kerala
10.	Aswathy	Single plant selection from somaclones of cultivar Rio-de-Janeiro. Released from KAU, Thrissur, Kerala
11.	KAU Chandra	Somaclone (SE 8681) from KAU,Thrissur, Kerala
12.	Mohini	Clonal Selection (GCP 49), Released from UBKV, Pundibari, West Bengal
Local types		
13.	Rio de Janeiro	Popular ginger cultivar in South India
14.	Nadia	Landrace from West Bengal
15.	Maran	Landrace from Assam
16.	Himachal	Landrace from Himachal Pradesh
17.	Bhaise	Common cultivar of ginger in North-East India, collected from Sikkim
18.	Gorubathane	Popular cultivar in Assam and West Bengal, collected from Sikkim
19.	Mahim	Local cultivar, collected from Zaheerabad, Telangana
20.	Zaheerabad local	Local cultivar, collected from Zaheerabad, Telangana
21.	Arunachal Pradesh local	Local cultivar, collected from Arunachal Pradesh
Distinct types		
22.	Red ginger (*Zingiber officinale* var. *rubra*)	Sourced from National Active Germplasm Site (NAGS), ICAR-Indian Institute of Spices Research, Kerala
23.	Black ginger (*Kaempferia parviflora*)	Sourced from National Active Germplasm Site (NAGS), ICAR-Indian Institute of Spices Research, Kerala
24.	Mango ginger (*Curcuma amada*)	Sourced from National Active Germplasm Site (NAGS), ICAR-Indian Institute of Spices Research, Kerala
Promising lines		
25.	Acc. 247	Promising germplasm collections, Sourced from National Active Germplasm Site (NAGS), ICAR-Indian Institute of Spices Research, Kerala
26.	Acc. 65	
27.	Acc. 578	
28.	Acc. 219	
29.	Acc. 833	
30.	RG 3	Sourced from National Active Germplasm Site (NAGS), ICAR-Indian Institute of Spices Research, Kerala

3.4 Experiment I –Studies on genetic diversity in ginger

3.4.1 Morphological characterization

Morphological characterization was done based on DUS guidelines (PPV&FRA, 2007).

Test plot design

Design	:	RCBD
No. of genotypes	:	30
Bed size	:	3 m^2 (3 m x 1 m)
Spacing	:	30 x 25 cm
Plants per replication	:	40
Number of replications	:	2
Seasons	:	2 (*Kharif* 2016-17 and *Kharif* 2017-18)

3.4.1.1 Planting

Land was ploughed to fine tilth and beds of 3 m^2 (3 m x 1 m) were prepared. Rhizome pieces of 20-25 g having one or two good buds were used as source of planting material. Before planting, rhizomes were treated with 0.3% mancozeb for 30 minutes.

Plate 1: General view of ginger experimental field during 2016-17

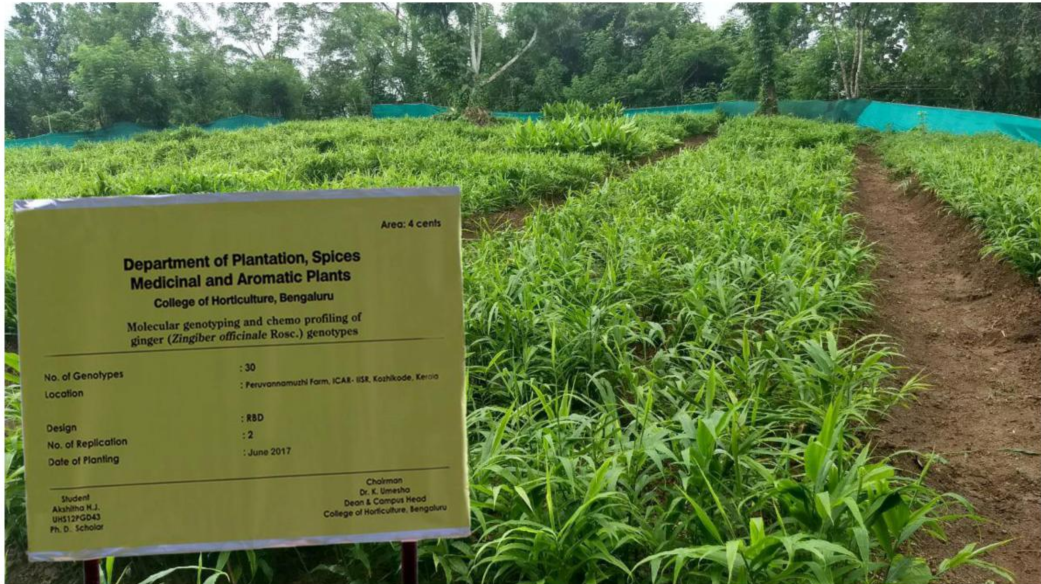


Plate 2: General view of ginger experimental field during 2017-18

Rhizomes were planted at spacing of 30 x 25 cm and rhizome bits were placed in shallow pits and are covered with thin layer of soil. Mulching was done with green leaves (Jayashree *et al.,* 2015). General view of experimental field during 2016-17 and 2017-18 (Plate 1 & 2).

3.4.1.2 Aftercare

3.4.1.2.1 Irrigation

Crop was cultivated as rainfed crop.

3.4.1.2.2 Fertilizer application

Fertilizer application was done as per the standard package of practice of IISR which was 70:50:50 kg/ha. Fertilizers were applied at 45 and 90 days after planting. Foliar application of ginger micronutrient mixture (dosage @ 5 g/l) developed by IISR was sprayed twice at 60 and 90 DAP.

3.4.1.2.3 Intercultivation

Weeding was done just before application of fertilizers and immediately after fertilizer application beds were mulched with green leaves. Earthing up which is essential to prevent exposure of rhizomes was done after weeding and fertilizer application.

3.4.1.2.4 Pest and disease management

Need based pest and disease management measures were taken up as and when required.

3.4.1.3 Observations recorded

Observations were recorded for two consecutive years as per the DUS guidelines of PPV&FRA. Morphological characters were recorded at the end of the growth phase *i.e.,* 150 days after planting and rhizome characters were recorded after harvest. Following observations were recorded from five randomly tagged plants from each replication

3.4.1.3.1 Growth habit

Growth habit of the plant was recorded by visual observation and grouped as erect, semi erect or spreading.

3.4.1.3.2 Plant height (cm)

Plant height (cm) was measured from soil level to tip of the top leaf of the main shoot. Genotypes were grouped as short (<100 cm), medium (100-120 cm) or tall (>120 cm) based on these observations.

3.4.1.3.3 Number of shoots

Total number of shoots in a single clump was counted. Based on the observation, genotypes were grouped as few (<10), medium (10-15), many (>15) shoots.

3.4.1.3.4 Shoot diameter (cm)

Diameter (cm) of the tallest pseudostem of each clump was recorded. Based on shoot diameter grouping was done as narrow (<3 cm), medium (3-5 cm) and broad (>5 cm).

3.4.1.3.5 Number of leaves on main shoot

Number of leaves on the main shoot of the clump was recorded and grouping was done as few (<25), medium (25-35) and many (>35).

3.4.1.3.6 Total number of leaves

Total number of leaves present in a clump was recorded.

3.4.1.3.7 Leaf length (cm)

Length of upper fourth leaf from the main shoot was recorded and grouping was done as short (<25 cm), medium (25-30 cm) and long (>30 cm).

3.4.1.3.8 Leaf width (cm)

Width was also recorded from the upper fourth leaf of the main shoot. Based on width, genotypes were grouped as narrow (<2.5 cm), medium (2.5-3.5 cm) and broad (>3.5 cm).

3.4.1.3.9 Leaf area

Leaf area was calculated as per the model developed by Kandiannan *et al.*(2009).

$$\text{Leaf area (LA)} = -0.0146 + 0.6621 \times L \times W$$

Where LA is leaf area (cm^2), L is leaf length (cm) and W is the maximum width of the leaf (cm)

3.4.1.3.10 Rhizome thickness (cm)

Rhizome thickness (cm) was recorded after the harvest. Observation was recorded on the primary rhizome by cutting the rhizome. Genotypes were grouped based on this as thin (<2 cm), medium (2-3 cm) and bold (>3 cm).

3.4.1.3.11 Rhizome shape

Rhizome shape was recorded after harvest by visual observation and based on rhizome shape, genotypes were grouped as straight, curved and zigzagged.

3.4.1.3.12 Dry recovery (%)

Dry recovery was recorded after drying. Fresh rhizomes were peeled and sun dried to record the dry recovery (%). Based on this genotypes were grouped as low (<16%), medium (16-18%) and high (>18%).

3.4.1.3.13 Yield per plant (g)

Yield was recorded after the harvest by weighing individual clump.

3.4.1.3.14 Projected yield (t/ha)

Based on the yield per plant and yield per bed, projected yield (t/ha) was calculated.

For mango ginger and black ginger, turmeric DUS guidelines (PPV&FRA, 2007) were followed for grouping.

3.4.1.4 Statistical analysis

3.4.1.4.1 Study of variability parameters

3.4.1.4.1.1 Analysis of variance (ANOVA)

The analysis of variance for different characters was carried out using mean data in order to assess the genetic variability among genotypes as given by Cochran and Cox (1957). The level of significance was tested at 5 % and 1 % using F test.

3.4.1.4.1.2 Estimation of genetic variability

1. Genotypic and phenotypic variance

Phenotypic and genotypic components of variance were estimated by using the formula given by Cochran and Cox (1957).

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

Phenotypic variance (σ^2_p) = Genotypic variance (σ^2_g) + Environmental variance (σ^2_e)

2. Phenotypic and genotypic coefficient of variation

Phenotypic and genotypic coefficients of variation for all characters were estimated using the formula of Burton and De Vane (1953).

$$\text{Genotypic Coefficient of Variation (GCV\%)} = \frac{\sqrt{\text{Genotypic variance}}}{\text{Grand mean}} \times 100$$

$$\text{Phenotypic Coefficient of Variation (PCV\%)} = \frac{\sqrt{\text{Phenotypic variance}}}{\text{Grand Mean}} \times 100$$

PCV and GCV were classified as per Sivasubramanian and Menon (1973) as given below:

0-10%- low; 10.1-20%- moderate; >20.1%- high

3. Heritability in broad sense (h^2)

The broad sense heritability (h^2_{bs}) was estimated for all the characters as suggested by Lush (1949).

$$\text{Heritability} = \frac{\text{Genotypic variance}}{\text{Phenotypic variance}} \times 100$$

Heritability was classified as suggested by Robinson *et al.* (1949) and described as 0-30 %: Low, 30-60 %: Moderate, 60 % and above as High.

4. Genetic advance

Genetic advance for each of the character was calculated by using the formula given by Johnson *et al.* (1955).

$$GA = \frac{h^2 \times \sigma_p \times K}{100}$$

Where, h^2 = heritability (broad sense)

σ_p= Phenotypic standard deviation

K = Standard selection differential (2.06 @ 5% selection intensity)

Genetic advance as per cent mean was estimated by using the following formula

$$\text{Genetic advance as per cent mean} = \frac{\text{Genetic advance}}{\text{Mean}} \times 100$$

The genetic advance as per cent mean was categorized into 0-10%: Low, 10-20 %: Moderate, 20 % and above: High as suggested by Johnson *et al.* (1955).

3.5 Experiment II - Development of chemical profiles

3.5.1 Oleoresin (%)

Dried and powdered rhizome samples were used for extraction of oleoresin. Oleoresin estimation was done using ASTA (1978) method. Ten gram of sample was weighed and transferred to glass column (18 × 500 mm) with stopcock. To this 50 ml of acetone was added and allowed to stand overnight. Filtrate was obtained through non absorbent cotton and collected in pre-weighed 100 ml beaker. Next day acetone was filtered and 30 ml of acetone was added and allowed to stand for 1 hour. Then the filtrate was kept on water bath to evaporate the acetone. Beakers were kept in oven until constant weight was obtained. The amount of oleoresin was estimated gravimetrically (ASTA, 1978).

$$\text{Oleoresin (\%)} = \frac{\text{Weight of residue (g)}}{\text{Weight of sample (g)}} \times 100$$

3.5.2 Crude fibre (%)

The crude fibre content was estimated in the powdered ginger sample using Fibra plus FES 6 from Pelican equipment. The method followed was as per ASTA (1978).

The empty crucibles were weighed and added with 1 g of coarsely ground dry sample. Crucibles were attached to extraction unit. Digested the sample with 200 ml of 1.25 per cent sulphuric acid solution at 500 °C until it began to boil, then temperature was set to 400 °C and allowed to boil for 45 minutes. Drained the acid and washed the samples with distilled water 2-3 times. Then, alkali wash was done with 1.25 per cent sodium hydroxide, similar to acid wash. Then washed with distilled water for 2-3 times. Crucibles were then dried in hot air oven until the crucibles were free from moisture. Crucibles were cooled to room temperature and recorded their weight until constant value CWBA (W_1). The crucibles were kept in muffle furnace at 500 °C till ash became white and record the final weight CWAA (W_2).

$$\text{Crude fibre (\%)} = \frac{W_3}{W} \times 100$$

$W_3 = W_1 - W_2$; W = Sample weight (1 g)

3.5.3 Essential oil (%)

Essential oil was extracted using Clevenger method (ASTA 1978). Twenty gram of powdered sample was weighed and transferred to one litre short neck round bottom flask with 500 ml of water. Apparatus was set in the Clevenger apparatus and boiled for 3 hours. Allowed to stand until the oil layer was clear. The volume of the oil collected was noted (nearest 0.02 ml). Kept overnight and transferred the oil to new 1 ml eppendorff tube, added a pinch of anhydrous sodium sulphate to remove traces of water and stored at 4 °C for GC/MS analysis.

$$\text{Volatile oil (V/W) (\%)} = \frac{\text{Amount of oil collected (ml)}}{\text{Weight of sample (g)}} \times 100$$

3.5.4 Essential oil yield per unit area (l/ha)

Essential oil yield per unit area (l/ha) was calculated by converting the essential oil percentage to essential oil yield per hectare on dry weight basis.

3.5.5 GS/MS analysis

3.5.5.1 Instrument condition

Ginger oil was analyzed using Shimadzu GC-2010 gas chromatograph equipped with QP 2010 mass spectrometer. RTX-Wax column (30 m × 0.25 mm, film thickness 0.25 µm) was used. Helium was used as the carrier gas at a flow rate of 1.00 ml min^{-1}. The injection port was maintained at 250 °C; the detector temperature was 220 °C and the oven temperature was programmed as follows: 60 °C for 5 minutes and then increased to 110 °C at the rate of 5 °C per minute, then up to 170 °C at the rate of 3 °C per minute, again up to 220 °C at the rate of 5 °C per minute, at which the column was maintained for 3 minutes. The split ratio was 1:40. Mass range was 40-650 amu and the scan speed was 1250. Total run time was 48 minutes. The constituents of the oil were identified by a comparison of retention indices with those reported in literature, by matching the mass spectral data with those stored in Nist and Wiley library.

3.6 Experiment III – Development of DNA profiles of ginger genotypes

3.6.1 Molecular characterization

3.6.1.1 DNA isolation

Young leaves from 45-60 days old plants were selected for DNA isolation. Leaves were wiped and one gram of leaf was taken for DNA isolation.

3.6.1.1.1 Extraction procedure

Genomic DNA was isolated using the CTAB method (Syamkumar *et al.,* 2003). The procedure followed is given below

1. One gram of cleaned leaf tissue was ground in liquid nitrogen using pre chilled pestle and mortar. While grinding a pinch of Polyvinylpyrrolidone (PVP) was added.
2. The powder was transferred to 50 ml Oakridge tube and add 10 ml preheated CTAB buffer.
3. 30 µl of mercaptoethanol was added and the samples were incubated at 65 °C for 1 hour with occasional mixing by gentle swirling.
4. Tubes were kept to attain room temperature.
5. Equal volume of Chloroform: Isoamyl alcohol (24:1) was added and mixed by gentle swirling. Spin at 12000 g for 10 minutes at 4 °C.
6. Aqueous phase was transferred to a fresh tube with cut tips.
7. 2/3 volume of Isopropanol was added and mixed gently. Kept at room temperature for 30 minutes.
8. Spinned at 8000 g for 5 minutes and flow through was discarded.
9. To the pellets 1 ml of 70% ethanol was added and spinned at 8000 g for 2-5 minutes, supernatant was discarded and vacuum dried for 20 minutes.
10. Dissolved in 100 µl of TE buffer/nuclease free water.

3.6.1.1.2 Purification procedure

1. 3 $\mu g ml^{-1}$ of RNase was added and incubated at 37 °C for 1 hour.
2. Equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) was added and mixed well.

3. Spinned at 14000 g for 10 minutes at 4 °C. Aqueous phase was transferred to fresh microfuge tube.
4. Extraction was done with equal volume of chloroform:isoamyl alcohol (24:1), spinned at 10000 rpm and transferred the aqueous phase to fresh tubes.
5. Equal volume of 100 % ethanol was added & mixed gently and incubated at 4 °C for 1 hour.
6. Centrifuged at 8000 g for 5 minutes.
7. Supernatant was decanted carefully and pellets were washed with 70 % ethanol.
8. Pellets were vacuum dried and dissolved in 500 μl of TE buffer/nuclease free water.

3.6.1.2 Quantification of DNA

Quantification of DNA was carried out by gel based quantification.

3.6.1.2.1 Agarose gel analysis

Gel based quantification was carried out by visualizing the concentration of DNA in 0.8 % agarose gel along with a standard DNA marker. Quantity of DNA was estimated by comparing the intensity of the DNA band of the sample with that of the standard DNA marker.

3.6.1.2.1.1 Preparation of agarose gel

- Gel tray was cleaned and fixed the tapes on both the ends. Tray was kept horizontally and combs were kept.
- In 100 ml of 1X TBE buffer 0.8 g of agarose was added and boiled in microwave oven until all the agarose particles completely dissolved. Kept it for cooling.
- To the cooled gel 1-2 μl of ethidium bromide was added and was poured to gel tray.
- After gel solidifies tape and the comb were removed, gel was placed in the tank and 1X TBE buffer was poured until the gel is fully immersed.
- DNA samples were loaded with loading dye (6 X) into the wells with standard DNA marker.
- Gel was run at 80 volts. Gel was documented using gel documentation system.

3.6.2 Polymerase chain reaction

RAPD and SSR primers were used for the amplification of isolated DNA.

3.6.2.1 RAPD analysis

RAPD analysis was done as follows

3.6.2.1.1 PCR components for RAPD analysis

- Assay buffer (10X with 15mM $MgCl_2$) - Genei, India
- dNTPs (10 mM μl^{-1}) – Genei, India
- Primers (10 mM μl^{-1}) – Sigma Aldrich
- Taq DNA polymerase (3U μl^{-1}) - Genei, India
- Template DNA (30-40 ng μl^{-1})

3.6.2.1.2 PCR protocol for RAPD

PCR components		Volume (µl)
Nuclease free water	:	14.4
10 X reaction buffer with 15mM $MgCl_2$	:	3.5
dNTP mix (10mM μl^{-1})	:	3.0
Primer (10 mM μl^{-1})	:	2.0
Template DNA	:	1.6
Taq DNA polymerase (3 U μl^{-1})	:	0.5

Table 2: RAPD primers used for analysis

Primer	Sequence (5' – 3')
OPA-03	AGT CAG CCA C
OPA-05	AGG GGTC TTG
OPA-08	GTG ACG TAG G
OPA-09	GGG TAACGC C
OPA-10	GTGATCGCAG
OPA-17	GAC CGC TTG T
OPA-18	AGG TGA CCG T
OPB-08	GTC CAC ACG G
OPB-20	GGA CCC TTA C
OPC-09	CTC ACC GTC C
OPC-20	ACT TCG CCA C
OPD-03	GTCGCCGTCA
OPD-07	TTG GCA CGG G
OPD-13	GGG GTG ACG A
OPD-14	CTTCCCCAAG
OPD-16	AGG GCG TAA G
OPD-18	GAG AGC CAA C
OPD-20	ACC CGG TCA C
OPE-11	GAG TCT CAG G
OPF-02	GAG GAT CCC T
OPF-09	CCA AGC TTC C
OPG-11	TGCCCGTCGT
OPH-08	GAA ACA CCC C
OPH-15	AATGGCGCAG
OPI-07	CAG CGA CAA G
OPJ-16	CTG CTT AGG G
OPL-12	GGG CGG TAC T
OPQ-08	CTC CAG CGG A
OPU-17	ACCTGGGGAG
OPW-11	CTGATGCGTC

Thirty randomly selected RAPD primers were used in the study

3.6.2.1.3 PCR programming

Initial Denaturation at 94 °C for 3 minutes
Denaturation at 94 °C for 45 seconds
Annealing at 37 °C for 45 seconds   35 cycles
Extension at 72 °C for 1 minute
Final extension at 72 °C for 15 minutes
Storage at 4 °C

3.6.2.2 SSR analysis

SSR reactions were performed as follows

3.6.2.2.1 PCR components for SSR analysis

- Assay buffer (10X with 15mM $MgCl_2$) - Genei, India
- dNTPs (10 mM μl^{-1}) - Genei, India
- Primers (10 mM μl^{-1}) - Sigma Aldrich
- Taq DNA polymerase (3U μl^{-1}) - Genei, India
- Template DNA (30-40 ng μl^{-1})

3.6.2.2.2 PCR protocol for SSR

PCR component		Volume (µl)
Nuclease free water	:	11.8
10 X reaction buffer with 15mM $MgCl_2$	:	2.5
dNTP mix (10mM μl^{-1})	:	2.0
Forward primer (10 mM μl^{-1})	:	1.0
Reverse primer (10 mM μl^{-1})	:	1.0
Template DNA	:	1.5
Taq DNA polymerase (3 U μl^{-1})	:	0.2

Final volume of PCR product was made upto 20 µl

Table 3: SSR primers used for analysis

Primer	Sequence (5' – 3')	Annealing temperature (T_a) °C
EST SSR primers		
ZOC 5	GGAGTATCTTCACCTCTGTGCC ACCCTCACCTTCTCCAAGC	60
ZOC 11	GCTGCTGGTACTTGGCTTTC CTCCTTCCTTTGCCTATCAAGA	60
ZOC 22	GGAGATTGGGTTCATCGTCTAC AGCAAAGAAATAGGACAAGGCA	60
ZOC 28	GCCTTCTTCGGAGTGTCCTT AACCAAAGCCTAATCCAAAACC	60
ZOC 40	CAAGAAGCCCGTTGTCATTT TTCAACAAACACGGAACAGAAG	60
ZOC 44	TGCAGAGAATAGGACAGAACCC ACGGCACATCACTTACTTAGACAC	60
ZOC 49	CGTCGAACAATACCTCTCCTTC TCAATCTCATGTCCAAGTACGC	60
ZOC 56	CAACAGCGTCACCATCTGAAT TCGTTCTCATCGAACCACG	61
ZOC 60	GAAGCGACCAATCAACAACC GGCAACGATGGTGTGTGTATAA	60
ZOC 83	CCTTTTGTATTTCCATCGTCGT AAAACGCCTCTTGTTCTCCAT	60
ZOC 86	CACTTTGTCTGAACGATTCCCT TGGTGGTGGTGTAGTCTTCTTG	60
ZOC 91	GCAGATCCAGAACTCCACCTAA ATTTCCTCTGTTCCATCTCAGC	60
ZOC 92	GTAGTCCCCAAACAGAAACTCG AGATCGAGGTGGTCAGCAAT	60
ZOC 98	TGCCACTCAATAACATGAACC CGATACATAACAAGCAAGCAAC	58
ZOC 99	GCTCTTGTATCTCCATTTTGGG GCTCCCCTGGCTCTTGTTA	60
ZOC 100	CATCCCACTGGAAGCGTACAAAC AGGTCGGAGGTGAAGTCTCTG	61
ZOC 102	TCTCGGGCACCAAAATAAATAC TCATCGTGTTGTAGAAAATGGC	60
ZOC 106	CGAGAAATTGCAGCCAAAA CCAACGAGTTCTTCAACATCAA	60
ZOC 152	CCTGACTTAGCTTCCTTCATCG GTAGTTGGCATCGAGGGTCAT	61

Primer	**Sequence (5’ – 3’)**	**Annealing temperature (T_a) °C**
ZOC 156	GAGGACAAGGAAGGGACAGG GAGTTCGATTTGGTCTGAGGAG	61
ZOC 179	CACGCTGTCATTGCGATCT AATCAAGAGCAGGAACACGAAC	60
ZOC 186	TCGACACCACCACCAGAACAGAAT CAATCACTCTTGCATCTTCACC	60
Genomic SSR primers		
GB-ZOM-033	CAGCAGATTTTTGCTCCG GTCGCGTTCGTGGAAAT	55
GB-ZOM-040	TCTCCCTCTCGGATCCAT ATCCATTGCCTGATGGTG	60
GB-ZOM-055	GTGAGCAGAAAACAGCCG TCGCCAATTGAAGACCAC	58
GB-ZOM-064	CGTAGGATCTTCCCGACC CGAGTGAACCCATGGAGA	60
GB-ZOM-103	GCTGCGGACTAAATGCTG ACGCTAGGGAACAGGGAG	63
GB-ZOM-107	TCCAAAGGTGCTGTTGCT CTGTGTTTTTCTTCGCCG	58
GB-ZOM-111	TAACCGGGAGAAAACCGT ACTCGTCCGATTCCGATT	52
GB-ZOM-140	AGGGGGCAGTGGAGAG ACGTTCCTGCACTTGACG	55
Primers from *Curcuma longa*		
CuMiSat-01	AAACCGCAAGAAAACTGAAG CTCTTCCCTGAACGATTCC	62
CuMiSat-03	GCACTACTTCCTTCTCGTTCAA CGTCGTAAAGATTAGCGTGTG	65
CuMiSat-06	AAGAAACTCCAACCACAATCC CTTGTCTTCCTCCTCCATTG	62
CuMiSat -10	CACCCTATGAGTGCTAACTGAAG ACCTGCACCACGATCAAC	65
CuMiSat -11	ACAGTCCCCTTCCCACTC TCTTGTTCCTATGCTCTACGC	65
CuMiSat -13	CCCGAAGCCATTTCTCAG TCGTCTCTCCTCTGCCAAC	65
CuMiSat -15	GCAGAACTCACCAAGTAATGGC TTGAACAACCAACACCCTAACTG	62
CuMiSat -19	CATGCAAATGGAAATTGACAC TGATAAATTGACACATGGCAGTC	65

Primer	Sequence (5' – 3')	Annealing temperature (T_a) °C
CuMiSat -20	CGATACGAGTCCATCTCTTCG CCTTGCTTTGGTGGCTAGAG	65
CuMiSat -21	TCATTCAAAGTCCGATGGAA TTCGAGTGCAGAAGGAGAATTA	62
CuMiSat -23	CGTGGAAGGTGAGTTTGAC CAGAAGGGAACTGAGATGG	65
CuMiSat -28	TTCAACTTCTCCTCGCTCAG GCAAGGTCTGCATCTATTTCTC	65
CuMiSat -29	GTGGTATCCCCATGAAGAGC ATGACCAAGCCCTTTCACC	65
CuMiSat -30	CTCTAATGTCGCCTCTCACG GCATCTCCCGTTCTTCTCC	65
CuMiSat -31	GGAGGAGGAGAAGCAGAAG GACAGGCGAAGGAAGAAAC	65
CuMiSat -33	ATGGATGGATACAACAACAAC TATAAACACACTCCCTCTTGG	65
CuMiSat -34	AAGTTGGTGAAGGATTAGAGCTAC CACCTAGTGGGATAAATCTTGG	62
CuMiSat -35	GGTTCGTCGCTGGAAAGTAAT GCATCTCAACAGGGGCTG	60
CLEST 10	GTGGTGGAGGAGGAAGAGAAG TTGAGGGAACAAAAGGAAGAC	65
CLEST 11	TTCATTCGACGCAAACAGC CGACGCAATAGTCGAAGGC	65
CLEST 12	GGGATTGAGGTGGAGGTAGG GCTGGCGAAGTAGAAGAAGAAG	65
CLEST 13	TGTACAAGCTCCAAATAAGTCAAG CAGGAGTGTTCTAATGTTGCCC	65
CLEST 14	CACCTCTCCTTCCCCAACC GCCGTCCTCGCTTCTTCTTA	65
CLEST 15	GCCAAAGAAAGAACTGACATCC TTACAACCCTCCTCCCATTAGA	65
CLEST 16	AAGCAGTCCGTGGGAGAAG CTTCCTCAATCGAATGGCCG	65

3.6.2.2.3 PCR programming

Denaturation at 94 °C for 5 minutes
Denaturation at 94 °C for 45 seconds
*Annealing for 45 seconds — 35 cycles
Extension at 72 °C for 1 minute
Final extension at 72 °C for 20 minutes
Storage at 4 °C
*Annealing temperature varied between the primers

3.6.2.2.4 SSR primers

A total of 55 SSR primers were used in the present study *viz.,* 22 EST SSR primers (Anu Cyriac, 2016), eight ginger genomic SSR primers (Lee *et al.,* 2007) and 18 genomic SSR primers (Siju *et al.,* 2010a) & 7 EST SSR primers (Siju *et al.,* 2010b) from *Curcuma longa.*

3.6.2.3 Separation of alleles in agarose gel electrophoresis

The PCR products were checked in agarose gel stained with ethidium bromide. Agarose gel of 1.5 % was used for RAPD and 3 % agarose gel was used for SSR analysis.

3.6.2.4 Molecular diversity analysis

The independent as well as combined data generated for 30 genotypes from RAPD and SSR primers were subjected to statistical analysis.

3.6.2.4.1 Data analysis and data scoring

RAPD and SSR products were scored visually for presence (1) and absence (0) of bands. The scores were used to create a data matrix to analyze genetic relationship using the NTSYS-pc program version 2.02 (Exeter Software, New York, USA) described by Rohlf (1990). A dendrogram was constructed based on Jaccard's similarity coefficient (Jaccard, 1908) using the marker data from the ginger with unweighted pair group method (UPGMA).

Polymorphism information content (PIC) was calculated using the formula developed by Anderson *et al.* (1993). A PIC value of each locus was calculated as:

$$PIC_j = 1 - \sum_{l=1 \text{ to } L} P^2_{lj}$$

where P_{lj} is the relative frequency if the lth allele for the locus j and was summed up across all the alleles (L) over all lines. PIC provides an estimate of the discriminatory power of a locus by taking into account, not only the number of alleles that are expressed, but also the relative frequencies of those alleles. PIC values may range from 0 (monomorphic) to 1 (very highly discriminative), with many alleles in equal frequencies.

Genotypic gene diversity was calculated as described by Mariette *et al.* (2002).

$$H_g = 1 - (p_i^2 - q_i^2),$$

Where $p_i^2 - q_i^2$ are the frequencies of the dominant and null alleles, respectively. Here, allele frequencies were calculated based on the frequency of the null allele (*i.e.,* the number of individuals without the band). Where q_i represents the frequency of the null allele and p_i represents the frequency of the dominant allele,

$$q_i = \frac{(\text{No. of individuals for which the band was NOT present})^{1/2}}{\text{Total No. of individuals surveyed}}$$

$$p_i = 1 - q_i$$

3.6.3 Identification of SNPs from RNA-seq Data

3.6.3.1 Transcriptome sequences

SNPs (Single Nucleotide Polymorphisms) are genetic markers whose precise identification is prerequisite for SNP based diversity studies. Transcriptome data of five ginger genotypes retrieved from NCBI database were used for the SNPs identification. Details of the transcriptome sequences used are given below

Transcriptome sequence	Genotype	Source of data	Sequencing platform (Paired end)	Year
Genotype 1 (G1-HMCH)	Queensland	The University of Queensland, Australia	ILLUMINA (Illumina HiSeq 4000)	2018
Genotype 2 (SRR3268680)	IISR Varada	Indian Council of Agricultural Research (ICAR)-Indian Institute of Spices Research, Kozhikode, Kerala	ILLUMINA (Illumina Genome Analyzer)	2017
Genotype 3 (SRR1924263)	Maran	Rajiv Gandhi Centre of Biotechnology, Thiruvananthapuram, Kerala	ILLUMINA (Illumina Genome Analyzer IIx)	2016
Genotype 4 (SRR5313727)	Yujiang 1	Chongqing University of Art and Science, China	ILLUMINA (Illumina HiSeq 4000)	2017
Genotype 5 (SRR5512053)	Southwest	Chongqiong University of Art and science, China	Illumina HiSeq 2500	2018

3.6.3.2 SNP calling

Step 1: Filtering of raw reads

The raw reads of each transcriptome library were quality filtered by removing adapter sequences and read with quality score below Q20. The clean reads of the five libraries were de novo assembled using the Trinity program with default parameters (Grabherr *et al.*, 2011).

Step 2: Detection of variants in two different conditions

KisSplice is detecting SNP by a recognizable pattern, called a bubble formed in a *De Bruijn graph* (DBG) built from the reads (Sacomoto *et al.,* 2012 and Maestre *et al.,* 2016). KisSplice was carried out with default parameters with five conditions, using IISR Varada as reference to study SNPs of other four samples.

KisSplice Input
Queensland (G1-HMCH) V/s IISR Varada (SRR3268680)
Maran (SRR1924263) V/s IISR Varada (SRR3268680)
Yujiang 1 (SRR5313727) V/s IISR Varada (SRR3268680)
Southwest (SRR5512053) V/s IISR Varada (SRR3268680)

Step 3: ORF prediction by TransDecoder

TransDecoder software was used to predict the ORFs and the amino acid sequence of the transcripts. Based on TransDecoder prediction transcripts were classified as coding and non-coding RNA (when ORF is not predicted) (Maestre *et al.,* 2016).

Step 4: Mapping of SNPs by BLAT

BLAT was used to position the predicted SNPs onto the assembled transcripts (Maestre *et al.,* 2016; Kent, 2002).

Step 5: SNPs characterization

KisSplice2Reftranscriptome (K2RT) is a tool to assess the functional impact of SNPs detected using KisSplice based on location of SNP within transcript and amino acid change (Maestre *et al.,* 2016).

K2RT is done by using comparison of KisSplice, output of TransDecoder and reference transcriptome (IISR Varada). Output of K2RT will detect variants and classify them as:

SNP located within the non CDS - No ORF was predicted for the transcript

SNP located within the CDS and there is no change of amino acid - Synonymous

SNP located within the CDS and there is change of amino acid - Non synonymous

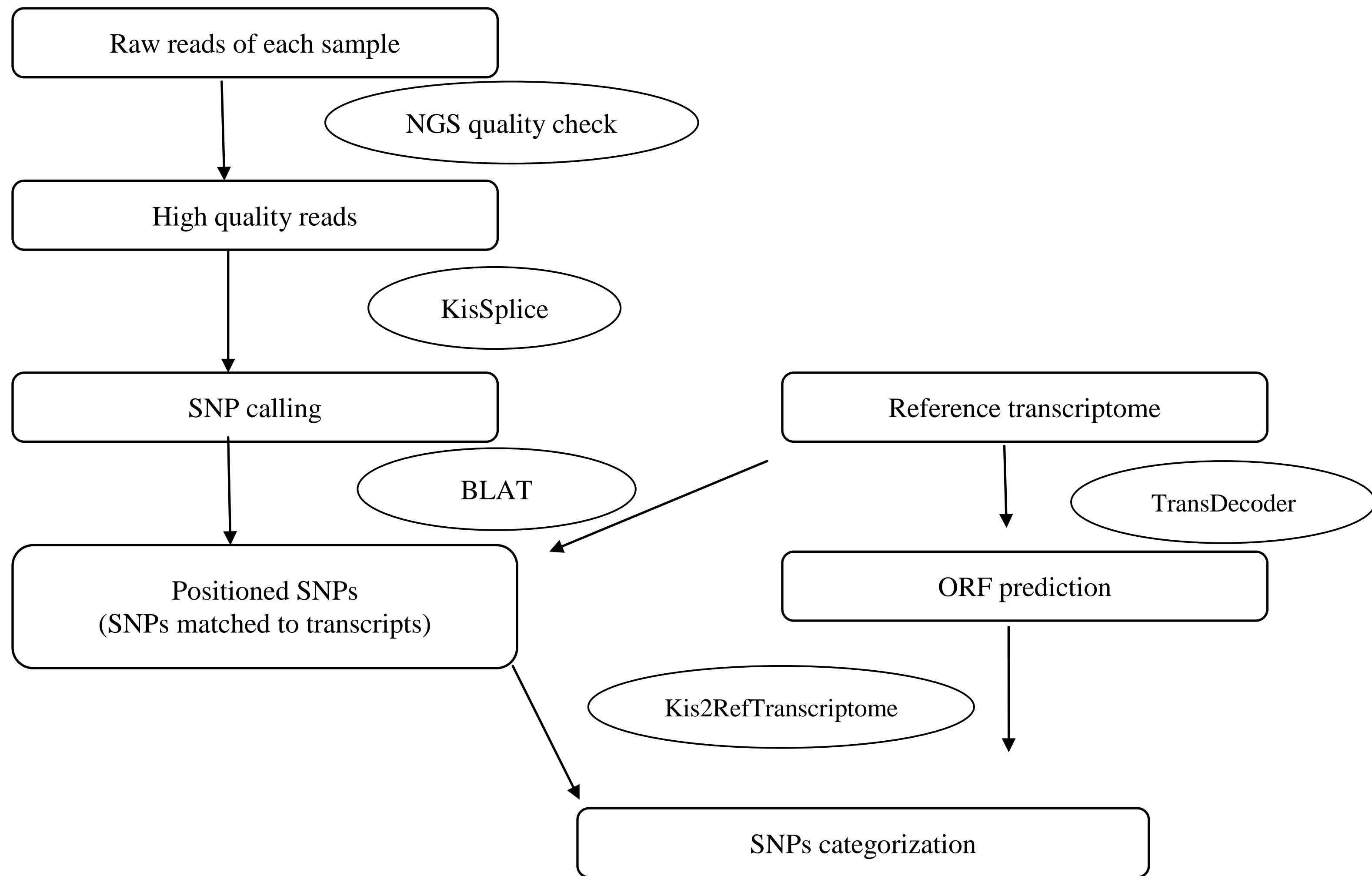


Fig. 1: Schematic representation of workflow of SNP identification

IV. EXPERIMENTAL RESULTS

The results obtained in the present investigation on “Molecular and chemo profiling of ginger (*Zingiber officinale* Rosc.) genotypes” are presented in this chapter.

4.1 Analysis of variance

The analysis of variance for Randomized Block Design for different characters is given in Table 4.

Significant differences were observed among the genotypes for all the characters except for leaf length. The significant differences for traits during both the years and also pooled data indicated that the genotypes had remarkable variation for different traits.

4.2 Studies on genetic diversity of ginger

4.2.1 Morphological characters of ginger genotypes

Morphological charactersof twenty eight ginger genotypes on eight morphological traits are presented in tables 5 & 6.

4.2.1.1 Plant height

Significant differences were observed in respect of plant height between the genotypes during both the years.

During 2016/17, highest plant height was recorded in Acc. 833 (69.90 cm) and lowest in Rio de Janeiro (48.00 cm). The genotypes Maran (68.62 cm) and IISR Varada (64.37 cm) were on par with Acc. 833.

During 2017/18, highest plant height was recorded in Suravi (78.60 cm) which was on par with Maran (77.40 cm), Gorubathane (75.90 cm), Nadia (74.30 cm), Bhaise (73.30 cm), Himachal (73.10 cm), IISR Varada (72.60 cm), IISR Rejatha (72.60 cm), Sourabh (68.90 cm) and KAU Chandra (67.7 cm). Lowest plant height was recorded in Red ginger (53.20 cm).

Table 4: Analysis of variance for different characters in ginger genotypes

Characters	2016-17			2017-18			Pooled		
	Mean sum of squares			Mean sum of squares			Mean sum of squares		
	Replication	Treatment	Error	Replication	Treatment	Error	Replication	Treatment	Error
	Degrees of freedom			Degrees of freedom			Degrees of freedom		
	1	27	27	1	27	27	3	27	81
Plant height (cm)	0.84	54.84**	9.89	147.87	113.54**	30.483	621.37	98.74**	36.67
Number of tillers	0.87	13.43**	0.71	0.02	17.14**	0.773	17.22	22.38**	3.23
Number of leaves on main shoot	0.04	7.47**	1.86	1.32	5.03**	1.201	419.87	7.65**	2.64
Total number of leaves	168.71	781.47**	38.81	282.60	899.87**	75.206	172.55	1094.49**	233.62
Leaf length (cm)	0.077	2.23	1.59	9.28	2.43	1.307	74.89	2.50	1.68
Leaf width (cm)	0.12	0.07*	0.03	0.05	0.087**	0.017	0.35	0.082**	0.045
Leaf area	20.41	22.09*	10.10	46.3	34.35**	10.301	377.43	30.61**	15.41
Shoot diameter (cm)	0.07	0.09*	0.04	0.19	0.16**	0.022	0.65	0.14**	0.067
Rhizome thickness	0.04	0.08**	0.01	0.03	0.13**	0.002	0.021	0.14**	0.022
Yield per plant (g)	1003.86	2717.22**	134.83	3.97	2564.86**	135.028	598.15	4627.77**	308.05
Projected yield (t/ha)	7.77	21.04**	1.04	0.02	19.86**	1.044	4.62	35.83**	2.38
Dry recovery (%)	6.99	21.088**	4.34	16.15	11.7**	4.517	14.67	26.07**	5.19
Essential oil (%)	0.007	1.63**	0.03	0.004	1.75**	0.009	0.96	3.13**	0.10
Oleoresin (%)	0.10	6.84**	0.07	0.016	7.01**	0.021	0.05	12.66**	0.43
Crude fibre (%)	0.82	7.01**	0.24	3.70	6.10**	0.283	1.56	13.07**	0.19

As per the pooled data, Maran recorded highest plant height of 73.01 cm and the lowest was observed in Rio de Janeiro (52.65 cm). The genotypes, Gorubathane (69.15 cm), IISR Varada (68.48 cm), Suravi (66.25 cm) and Nadia (65.50 cm) were found to be on par with Maran.

4.2.1.2 Number of tillers per clump

Highly significant differences were observed for number of tillers among the different genotypes during two experimental seasons.

Highest number of tillers per clump was recorded in Mahim (14.60) and lowest was in Mohini (4.90). Genotypes Arunachal Pradesh local (13.60), Rio de Janeiro (13.1) and Suprabha (13.00) were found to be on par with Mahim during 2016/17.

In the year 2017/18 also, Mahim put forth more number of tillers per clump (15.90) while it was lowest in Gorubathane (5.80). Genotype RG 3 (15.70) was at par with Mahim.

In the pooled data also, it was observed that, Mahim was having more number of tillers per clump (15.25) as like two years and genotypes Arunachal Pradesh local (13.60), Suravi (12.85) and Suprabha (12.75) were on par with Mahim. Less number of tillers per clump was observed in Acc. 578 (6.00).

4.2.1.3 Shoot diameter

Significant differences were observed in case of shoot diameter among different genotypes under study.

Significantly higher shoot diameter was observed in Maran (2.94 cm) and lowest was in Bhaise (2.11 cm) during 2016/17. Almost all the remaining genotypes were on par with Maran.

In the subsequent cropping season (2017/18), Aswathy produced highest shoot diameter of 3.41 cm and lowest was in Arunachal Pradesh local (2.15 cm).

Table 5: Variability in ginger genotypes for plant height, number of tillers, shoot diameter and number of leaves on main shoot

Variety/genotype	Plant height (cm)			Number of tillers			Shoot diameter (cm)			No. of leaves on main shoot		
	2016/17	2017/18	Pooled	2016/17	2017/18	Pooled	2016/17	2017/18	Pooled	2016/17	2017/18	Pooled
IISR Varada	64.37	72.6	68.48	7.0	8.1	7.55	2.69	3.08	2.88	18.5	15.3	16.90
IISR Mahima	59.79	64.9	62.34	9.1	8.9	9.00	2.85	2.47	2.66	16.3	11.4	13.85
IISR Rejatha	54.66	72.6	63.63	6.9	7.9	7.40	2.55	2.80	2.67	17.4	12.0	14.70
Suprabha	54.20	54.1	54.15	13.0	12.5	12.75	2.45	2.70	2.57	15.3	9.4	12.35
Suravi	53.90	78.6	66.25	10.4	15.3	12.85	2.85	3.05	2.95	19.2	9.7	14.45
Suruchi	53.90	66.3	60.10	11.0	11.2	11.10	2.7	2.75	2.72	16.8	8.9	12.85
Athira	62.00	61.1	61.55	6.6	9.0	7.80	2.85	2.75	2.80	19.2	9.4	14.30
Karthika	58.10	66.0	62.05	7.4	8.6	8.00	2.44	2.57	2.50	18.5	10.7	14.60
Aswathy	50.30	62.6	56.45	8.3	13.3	10.80	2.84	3.41	3.12	18.7	8.8	13.75
Sourabh	59.20	68.9	64.05	11.0	9.5	10.25	2.75	2.87	2.81	19.5	11.3	15.40
Mohini	62.90	66.0	64.45	4.9	8.9	6.90	2.5	2.70	2.60	17.5	10.8	14.15
KAU Chandra	54.10	67.7	60.90	6.9	7.4	7.15	2.55	2.68	2.61	15.2	11.1	13.15
Rio de Janeiro	48.00	57.3	52.65	13.1	7.9	10.50	2.3	2.87	2.58	15.5	10.2	12.85
Nadia	56.70	74.3	65.50	6.7	7.8	7.25	2.27	3.05	2.66	18.4	13.2	15.80
Maran	68.62	77.4	73.01	7.3	7.0	7.15	2.94	2.96	2.95	20.6	12.1	16.35
Himachal	52.21	73.1	62.65	6.7	9.6	8.15	2.4	2.89	2.64	14.4	11.0	12.70
Bhaise	53.86	73.3	63.58	8.7	9.5	9.10	2.11	3.28	2.69	16.7	9.0	12.85
Gorubathane	62.40	75.9	69.15	9.7	5.8	7.75	2.65	3.18	2.91	18.7	14.0	16.35
Mahim	52.40	55.1	53.75	14.6	15.9	15.25	2.4	2.40	2.40	16.9	8.8	12.85
Zaheerabad local	53.60	55.9	54.75	8.2	9.5	8.85	2.8	2.71	2.75	19.0	10.5	14.75
Arunachal Pradesh local	57.30	60.9	59.10	13.6	13.6	13.60	2.21	2.15	2.18	11.9	10.5	11.20
Acc. 247	56.40	62.8	59.60	6.6	5.8	6.20	2.47	2.55	2.51	18.8	10.5	14.65
Acc. 65	54.40	56.5	55.45	8.5	11.3	9.90	2.83	2.63	2.73	18.4	10.7	14.55
Acc. 578	53.40	60.2	56.80	5.2	6.8	6.00	2.53	2.85	2.69	15.8	9.5	12.65
Acc. 219	59.70	62.9	61.30	6.0	9.6	7.80	2.7	2.91	2.80	20.3	10.0	15.15
Acc. 833	69.90	56.7	63.30	6.8	13.3	8.63	2.61	2.80	2.70	18.0	11.9	14.95
RG 3	53.95	63.2	58.57	7.2	15.7	11.45	2.77	3.01	2.89	18.3	11.6	14.95
Red ginger	60.68	53.2	56.94	7.9	7.3	7.60	2.5	3.35	2.92	18.8	12.6	15.70
Mean	**57.17**	**65.18**	**61.09**	**8.54**	**9.89**	**9.22**	**2.59**	**2.83**	**2.71**	**17.59**	**10.88**	**14.24**
CD @ 5%	**6.45**	**11.33**	**8.52**	**1.73**	**1.81**	**2.52**	**0.44**	**0.31**	**0.34**	**2.80**	**2.25**	**2.28**
CV (%)	**5.50**	**8.49**	**9.91**	**9.91**	**8.91**	**19.49**	**8.46**	**5.25**	**9.09**	**7.76**	**10.06**	**11.41**

From the pooled data, it was evident that, genotype Aswathy was having more shoot diameter of 3.12 cm and less was in Arunachal Pradesh local (2.18 cm). The genotypes IISR Varada, Suravi, Athira, Sourabh, Maran, Gorubathane, Acc. 219, RG 3 and red ginger were on par with Aswathy.

4.2.1.4 Number of leaves on main shoot

During 2016/17 and 2017/18 highly significant differences were observed between the genotypes for number of leaves on main shoot.

In the first experimental season (2016/17), more number of leaves on main shoot was observed in Maran (20.6) and Arunachal Pradesh local recorded lowest number of leaves on main shoots (11.90). Genotypes which were on par with Maran were Acc. 219 (20.30), Sourabh (19.50), Suravi (19.20), Athira (19.20), Zaheerabad local (19.00), Acc. 247 (18.80), Red ginger (18.80), Aswathy (18.70), IISR Varada (18.50), Karthika (18.50), Nadia (18.40), Acc. 65 (18.40) and RG 3 (18.30).

During second experimental season (2017/18), more number of leaves on main shoot was recorded in IISR Varada (15.30) and lowest was in Aswathy (8.80). Genotypes Gorubathane (14.00) and Nadia (13.20) were found to be on par with IISR Varada.

The more and less number of leaves on main shoot was observed in IISR Varada (16.90) and Arunachal Pradesh local (11.20 cm) respectively which was indicated by pooled mean analysis. The genotypes Maran (16.35), Gorubathane (16.35), Nadia (15.80), Red ginger (15.70), Sourabh (15.40), Acc. 219 (15.15), Acc. 833 (14.95), Zaheerabad local (14.75), IISR Rejatha (14.70) and Acc. 247 (14.65) were found to be on par with IISR Varada.

4.2.1.5 Total number of leaves

During the experimental season’s *viz.,* 2016/17 and 2017/18 highly significant differences were observed among the twenty eight genotypes for total number of leaves in each clump.

Table 6: Variability in ginger genotypes for leaf characters

Variety/Genotype	Total no. of leaves			Leaf length (cm)			Leaf width (cm)			Leaf area (cm^2)		
	2016/17	2017/18	Pooled	2016/17	2017/18	Pooled	2016/17	2017/18	Pooled	2016/17	2017/18	Pooled
IISR Varada	80.8	84.8	82.80	17.88	20.30	19.09	1.97	2.07	2.02	23.34	27.87	25.60
IISR Mahima	94.2	81.1	87.65	17.60	21.85	19.72	2.45	2.13	2.29	28.47	30.77	29.62
IISR Rejatha	83.3	81.7	82.50	16.28	21.25	18.76	1.59	2.21	1.90	17.22	31.11	24.16
Suprabha	117.4	94.4	105.90	18.60	20.00	19.30	2.05	2.15	2.10	25.29	28.47	26.87
Suravi	121.1	133.8	127.45	17.90	19.40	18.65	1.95	1.85	1.90	23.14	23.75	23.44
Suruchi	109.3	81.1	95.20	18.70	22.45	20.57	2.00	2.47	2.23	24.86	36.71	30.78
Athira	81.6	72.8	77.20	17.30	22.40	19.85	1.72	2.25	1.98	19.63	33.36	26.49
Karthika	83.2	86.0	84.60	18.30	21.00	19.65	1.91	2.08	1.99	23.17	28.95	26.05
Aswathy	83.7	102.7	93.20	17.30	22.00	19.65	2.04	2.51	2.27	23.39	36.55	29.96
Sourabh	125.4	99.2	112.30	18.95	20.45	19.70	2.10	1.98	2.04	26.45	26.87	26.66
Mohini	57.7	83.0	70.35	16.70	20.25	18.47	1.70	1.98	1.84	18.78	26.47	22.62
KAU Chandra	70.2	80.1	75.15	18.20	21.50	19.85	1.99	1.97	1.98	24.02	28.11	26.06
Rio de Janeiro	111.6	74.2	92.90	16.20	20.55	18.37	1.91	2.06	1.98	20.56	28.03	24.29
Nadia	79.7	102.3	91.00	17.30	20.00	18.65	1.92	2.07	1.99	22.02	27.45	24.73
Maran	97.8	76.2	87.00	19.48	22.40	20.94	2.06	2.40	2.23	26.92	35.56	31.23
Himachal	73.7	95.8	84.75	19.70	20.50	20.10	2.25	2.15	2.20	29.36	29.36	29.36
Bhaise	84.1	76.6	80.35	17.80	21.60	19.70	1.98	2.64	2.31	23.36	37.77	30.56
Gorubathane	105.9	73.7	89.80	18.80	21.05	19.92	1.95	2.16	2.05	24.34	30.13	27.23
Mahim	125.5	128.8	127.15	17.55	18.70	18.12	1.87	1.92	1.89	21.75	23.81	22.78
Zaheerabad local	86	92.7	89.35	18.20	20.40	19.30	2.12	2.33	2.22	25.56	31.56	28.56
Arunachal Pradesh local	95.1	99.8	97.45	19.81	19.65	19.73	2.31	2.33	2.32	30.295	30.30	30.29
Acc. 247	76.3	55.7	66.00	17.60	23.30	20.45	1.85	2.43	2.14	21.58	37.46	29.52
Acc. 65	80.4	105.8	93.10	17.70	19.60	18.65	2.24	2.12	2.18	26.29	27.55	26.91
Acc. 578	49.9	49.4	49.65	18.20	20.85	19.52	1.99	2.02	2.00	23.99	27.94	25.96
Acc. 219	80.5	81.8	81.15	18.20	21.10	19.65	2.04	2.08	2.06	24.60	29.18	26.88
Acc. 833	62.6	125.5	94.05	17.90	20.00	18.95	2.13	2.35	2.24	25.33	31.12	28.22
RG 3	74.4	130.0	102.20	19.60	22.55	21.07	2.07	2.63	2.35	26.89	39.33	33.31
Red ginger	80.6	66.1	73.35	20.70	21.00	20.85	2.30	2.11	2.20	31.53	29.47	30.50
Mean	**88.28**	**89.82**	**89.05**	**18.15**	**20.93**	**19.54**	**2.01**	**2.19**	**2.10**	**24.36**	**30.53**	**27.44**
CD @ 5%	**12.78**	**17.79**	**21.50**	**NS**	**NS**	**NS**	**0.38**	**0.28**	**0.29**	**6.52**	**6.59**	**5.52**
CV (%)	**7.05**	**24.03**	**17.16**				**9.19**	**6.31**	**9.68**	**13.04**	**10.51**	**14.30**

In the year 2016/17, more number of leaves per clump was observed in Mahim (125.5) and less number of leaves was in Acc. 578 (49.90). On par results were observed in respect of Sourabh (125.40), Suravi (121.10) and Suprabha (117.40).

During 2017/18 maximum number of leaves per clump was recorded in Suravi (133.8) and genotypes such as RG 3 (130.00), Mahim (128.80) and Acc. 833 (125.50) were on par with Suravi. Whereas less number of total leaves on clump was observed in Acc. 578 (49.40).

Pooled data indicated that, more number of total leaves per clump was observed in the variety Suravi (127.45) and less number of leaves was observed in Acc. 578 (49.65). Genotypes Mahim (127.15) and Sourabh (112.30) were on par with Suravi.

4.2.1.6 Leaf length

Analysis of both the years data and pooled data on leaf length found to be non significant. Pooled analysis showed that, maximum leaf length of 21.07 cm was noticed in RG 3 and minimum was in Mahim (18.12 cm).

4.2.1.7 Leaf width

Leaf width ranged from 1.59 to 2.45 cm, highest leaf width was recorded in IISR Mahima (2.45 cm) whereas lowest in IISR Rejatha (1.59 cm) in the first cropping season. The genotypes Sourabh, Himachal, Zaheerabad local, Arunachal Pradesh local, Acc. 65, Acc. 833 and Red ginger were on par with IISR Mahima and RG 3 was at par with IISR Mahima.

During the second cropping season, Bhaise recorded highest leaf width of 2.64 cm and lowest leaf width was observed in Suravi variety (1.85 cm). The genotypes Suruchi, Aswathy, Maran, Acc. 247 and RG 3 were found to be on par with Bhaise.

Highest leaf width of 2.35 cm was recorded in RG 3 and lowest was in variety Mohini (1.84 cm), which was revealed by pooled mean analysis. The genotypes IISR Mahima, Suprabha, Suruchi, Aswathy, Maran, Himachal, Bhaise, Zaheerabad local, Acc. 247, Acc. 65, Acc. 219, Acc. 833 and red ginger were on par with RG 3.

4.2.1.8 Leaf area

Significant differences were observed among the genotypes in respect of leaf area during both the cropping seasons.

During 2016/17, leaf area of different genotypes ranged from 17.22 to 31.53 cm^2. Highest leaf area of 31.53 cm^2 was observed in red ginger and lowest was in IISR Rejatha (17.22 cm^2). The genotypes, Suprabha, Sourabh, Maran, Himachal, Zaheerabad local, Arunachal Pradesh local, Acc. 65, Acc. 833 and RG 3 were on par with red ginger.

In the year 2017/18, RG 3 recorded highest leaf area of 39.33 cm^2 and lowest was by Suravi (23.75 cm^2). Genotypes Suruchi, Athira, Aswathy, Maran, Bhaise and Acc. 247 were on par with RG 3.

Pooled mean analysis showed that, RG 3 was having the highest leaf area of 33.31 cm^2 and lowest was in Mohini (22.62 cm^2). IISR Mahima, Suruchi, Aswathy, Maran, Himachal, Bhaise, Zaheerabad local, Arunachal Pradesh local, Acc. 247, Acc. 833 and red ginger were on par with RG 3.

4.2.2 Rhizome characters and yield parameters of ginger genotypes

Rhizome and yield parametersof 28 ginger genotypes with respect to rhizome and yield traits are presented in table 7. Rhizomes of ginger genotypes presented in plates 3, 4, 5, 6 & 7.

4.2.2.1 Rhizome thickness

Highly significant differences were observed among the ginger genotypes with respect to rhizome thickness during both the years.

During both the years, genotypes with higher rhizome thickness were Bhaise (2.61 cm) and Himachal (2.54 cm) respectively and lowest rhizome thickness of 1.61 cm and 1.57 cm respectively was observed in Arunachal Pradesh local.

Pooled mean analysis of two years revealed that highest rhizome thickness was observed in Bhaise (2.55 cm) and the lowest was in Arunachal Pradesh local (1.59 cm).

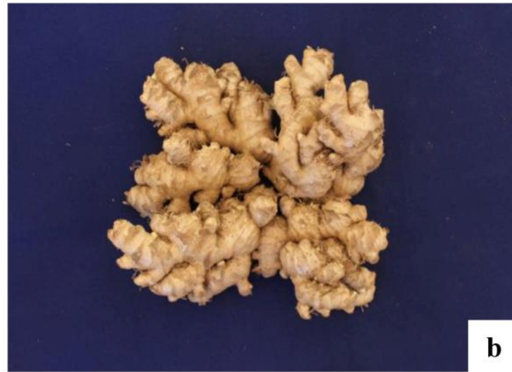


Plate 3: Rhizomes of ginger genotypes – a. IISR Varada; b. IISR Mahima; c. IISR Rejatha; d. Suprabha; e. Suravi; f. Suruchi

Table 7: Variability in ginger genotypes for rhizome and yield characters

Variety/Genotype	Rhizome thickness (cm)			Yield per plant (g)			Projected yield (t ha^{-1})			Dry recovery (%)		
	2016/17	2017/18	Pooled	2016/17	2017/18	Pooled	2016/17	2017/18	Pooled	2016/17	2017/18	Pooled
IISR Varada	2.16	2.17	2.16	167.50	192.90	180.20	14.74	16.97	15.85	22.10	23.18	22.63
IISR Mahima	2.11	2.29	2.20	104.50	106.50	105.50	9.19	9.37	9.28	24.46	19.09	21.77
IISR Rejatha	2.10	2.10	2.10	138.00	165.40	151.70	12.14	14.55	13.34	21.65	23.49	22.57
Suprabha	2.08	2.07	2.07	122.50	96.00	109.25	10.78	8.44	9.61	22.73	21.55	22.14
Suravi	2.30	2.10	2.20	132.00	95.20	113.60	11.61	8.37	9.99	23.72	23.19	23.46
Suruchi	2.11	2.11	2.11	141.00	115.62	128.31	12.40	10.17	11.29	23.42	24.50	23.96
Athira	2.05	2.00	2.02	154.50	133.40	143.95	13.59	11.73	12.66	24.08	24.42	24.24
Karthika	1.88	2.08	1.98	161.00	187.50	174.25	14.16	16.50	15.33	23.28	24.68	23.98
Aswathy	2.18	2.50	2.34	143.75	181.10	162.42	12.65	15.93	14.29	21.58	19.67	20.62
Sourabh	2.06	2.13	2.09	131.00	110.50	120.75	11.52	9.72	10.62	25.63	23.12	24.37
Mohini	2.13	2.28	2.20	145.50	124.00	134.75	12.80	10.91	11.85	24.02	22.29	23.15
KAU Chandra	2.21	2.08	2.14	139.00	170.75	154.87	12.23	15.02	13.62	26.15	23.33	24.74
Rio de Janeiro	1.86	1.82	1.84	108.00	117.20	112.60	9.50	10.31	9.90	18.69	16.33	17.51
Nadia	2.08	2.09	2.08	102.50	133.80	118.15	9.02	11.77	10.39	24.55	24.72	24.63
Maran	2.14	2.19	2.16	207.50	195.20	201.35	18.26	17.17	17.71	21.14	20.25	20.69
Himachal	1.88	2.54	2.21	197.00	168.06	182.53	17.33	14.78	16.06	25.42	21.85	23.64
Bhaise	2.61	2.49	2.55	185.00	152.62	168.81	16.28	13.43	14.85	18.46	19.75	19.10
Gorubathane	2.11	2.46	2.28	137.50	145.30	141.40	12.10	12.78	12.44	24.72	23.73	24.22
Mahim	1.69	1.73	1.71	151.50	133.87	142.68	13.33	11.78	12.55	20.92	19.11	20.01
Zaheerabad local	2.34	2.35	2.34	192.50	153.75	173.12	16.94	13.53	15.23	21.32	19.07	20.19
Arunachal Pradesh local	1.61	1.57	1.59	49.90	54.00	51.95	4.39	4.75	4.57	19.44	18.53	18.98
Acc. 247	2.27	2.46	2.36	195.15	176.16	185.66	17.17	15.50	16.33	20.94	20.49	20.71
Acc. 65	2.20	2.20	2.20	141.00	141.20	141.10	12.40	12.42	12.41	18.83	21.57	20.20
Acc. 578	2.08	2.23	2.15	122.50	144.80	133.65	10.78	12.74	11.76	27.86	24.40	26.13
Acc. 219	2.20	1.92	2.06	168.50	163.20	165.85	14.82	14.36	14.59	24.85	24.15	20.20
Acc. 833	2.47	1.58	2.02	159.50	157.70	158.60	14.03	13.87	13.95	17.62	21.00	19.31
RG 3	2.13	1.87	2.00	184.35	120.83	152.59	16.22	10.63	13.42	27.64	19.60	23.62
Red ginger	2.07	2.00	2.03	73.70	71.35	72.52	6.48	6.27	6.38	13.33	17.30	15.31
Mean	**2.11**	**2.12**	**2.11**	**144.87**	**139.56**	**142.21**	**12.74**	**12.28**	**12.51**	**22.45**	**21.58**	**22.01**
CD @ 5%	**0.25**	**0.13**	**0.25**	**23.82**	**23.84**	**24.69**	**2.09**	**2.09**	**2.17**	**4.28**	**4.35**	**3.20**
CV (%)	**5.85**	**3.08**	**8.08**	**8.01**	**8.33**	**12.31**	**8.01**	**8.32**	**12.32**	**9.28**	**9.84**	**10.34**

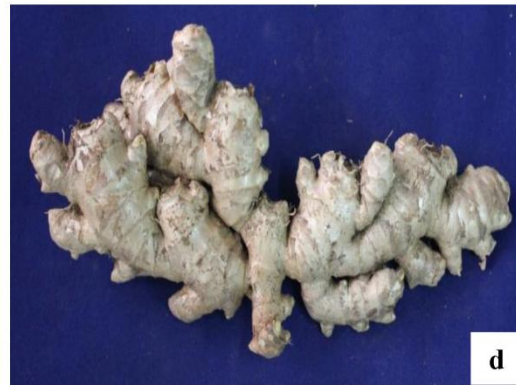


Plate 4: Rhizomes of ginger genotypes – a. Athira; b. Karthika; c. Aswathy; d. Sourabh; e. Mohini; f. KAU Chandra

The genotypes Aswathy (2.34 cm), Zaheerabad local (2.34 cm) and Acc. 247 (2.36 cm) were on par with Bhaise.

4.2.2.2 Yield per plant

Rhizome yield per plant exhibited highly significant differences among the genotypes during both the years.

Among the ginger genotypes under evaluation Maran recorded highest per plant yield of 207.50 g and lowest yield of 49.90 g plant^{-1} was recorded in Arunachal Pradesh local during 2016/17. Genotypes Acc. 247 (195.15 g plant^{-1}), Zaheerabad local (192.50 g plant^{-1}), Himachal (197.00 g plant^{-1}) and Bhaise (185.00 g plant^{-1}) were on par with Maran.

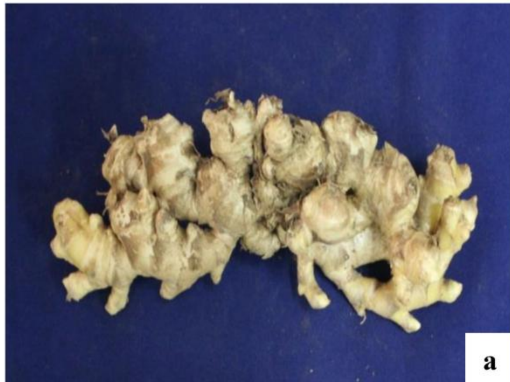
During the subsequent cropping season also similar trend was observed where the highest rhizome yield of 195.20 g plant^{-1} was recorded in Maran and lowest was in Arunachal Pradesh local (54.00 g plant^{-1}). Genotypes *viz.,* IISR Varada (192.90 g plant^{-1}), Karthika (187.50 g plant^{-1}), Aswathy (181.10 g plant^{-1}), Acc. 247 (176.16 g plant^{-1}) were on par with Maran.

From the pooled data, it is evident that the yield per plant was higher in Maran (201.35 g) and the lowest was in Arunachal Pradesh local (51.95 g plant^{-1}). The genotypes Acc. 247 (185.66 g plant^{-1}), Himachal (182.53 g plant^{-1}) and IISR Varada (180.20 g plant^{-1}) were on par with Maran.

4.2.2.3 Projected yield

Significant differences were observed among different ginger genotypes in terms of projected yield in both the years.

During 2016/17, highest yield of 18.26 t ha^{-1} was recorded in Maran and genotypes such as Himachal (17.33 t ha^{-1}), Acc. 247 (17.17 t ha^{-1}), Zaheerabad local (16.94 t ha^{-1}), Bhaise (16.28 t ha^{-1}) and RG 3 (16.22 t ha^{-1}) were on par with Maran. Lowest yield was in Arunachal Pradesh local (4.39 t ha^{-1}).



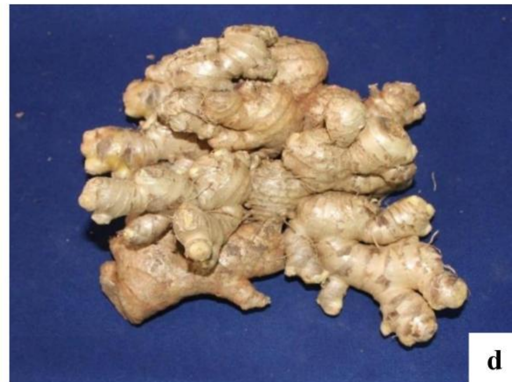


Plate 5: Rhizomes of ginger genotypes – a. Rio de Janeiro; b. Nadia; c. Maran; d. Himachal; e. Bhaise; f. Gorubathane

During 2017/18 also, Maran recorded highest projected yield of 17.17 t ha^{-1} and lowest was in Arunachal Pradesh local (4.75 t ha^{-1}). Genotypes IISR Varada (16.97 t ha^{-1}), Karthika (16.50 t ha^{-1}), Aswathy (15.93 t ha^{-1}), Acc. 247 (15.50 t ha^{-1}) and KAU Chandra (15.02 t ha^{-1}) were on par with Maran.

Pooled data of two years showed that highest projected yield of 17.71 t ha^{-1} was recorded in Maran and lowest was in Arunachal Pradesh local (4.57 t ha^{-1}). Genotypes Acc. 247 (16.33 t ha^{-1}), Himachal (16.06 t ha^{-1}) and IISR Varada (15.85 t ha^{-1}) were on par with Maran.

4.2.2.4 Dry recovery

Highly significant differences were observed among the genotypes in case of dry recovery.

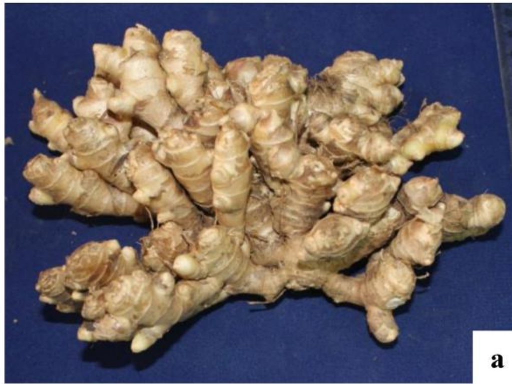
During 2016/17, highest dry recovery of 27.86 % was obtained in Acc. 578 and lowest was in red ginger (13.33%). Genotypes IISR Mahima, Suravi, Athira, Sourabh, Mohini, KAU Chandra, Nadia, Gorubathane, Acc. 219 and RG 3 were on par with Acc. 578.

Highest dry recovery of 24.72 % was obtained in Nadia and lowest (16.33 %) was obtained from Rio de Janeiro during 2017/18.

From the pooled mean analysis, it was noticed that the dry recovery was highest in Acc. 578 (26.13 %) and lowest was in red ginger (15.31 %). Genotypes KAU Chandra (24.74 %), Nadia (24.63 %), Gorubathane (24.22 %), Athira (24.24 %), Sourabh (24.37 %), Mohini (23.15 %), Suravi (23.46 %), Suruchi (23.96 %), Karthika (23.98 %), Himachal (23.64 %) and RG 3 (23.62 %) were on par with Acc. 578.

4.2.3 Components of variability for morphological characters

The estimates of range, general mean, coefficient of variation, heritability, genetic advance and genetic advance as per cent mean in respect of two year *viz.,* 2016/17,



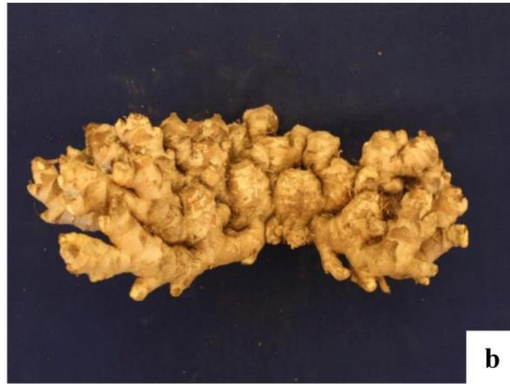


Plate 6: Rhizomes of ginger genotypes – a. Mahim; b. Zaheerabad local; c. Arunachal Pradesh local; d. Acc. 247; e. Acc. 65; f. Acc. 578

2017/18 and pooled data on different morphological characters are tabulated in Table 8 & 9.

4.2.3.1 Plant height

Low genotypic coefficient of variation for plant height was obtained during both the years. Similar pattern was observed for phenotypic coefficient of variation also. Estimates of heritability in broad sense were high (69.43 %) during 2016/17 and moderate (56.58 %) during 2017/18. Genetic advance as per cent mean was moderate during both the years.

Pooled analysis also revealed that, plant height exhibited low GCV of 9.12 % and moderate PCV of 13.47 %. Heritability in broad sense and as well as genetic advance as per cent mean was found to be moderate (45.84 % and 12.72 % respectively).

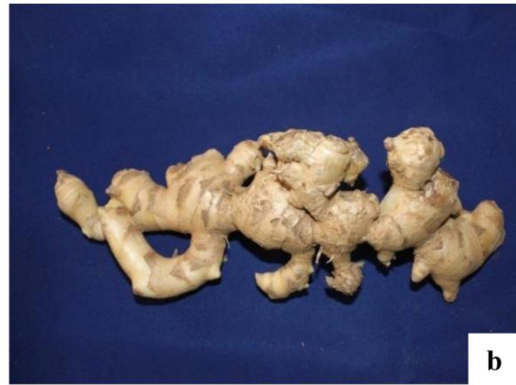
4.2.3.2 Number of tillers per clump

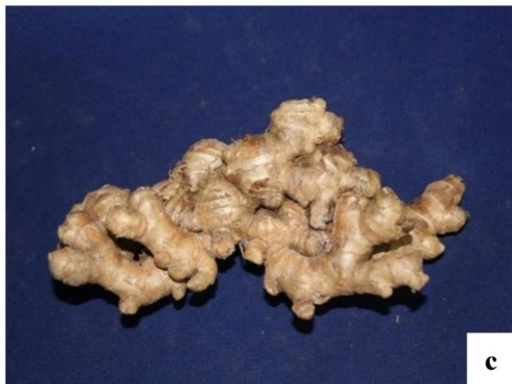
The highest GCV (29.50 %) and PCV (31.12 %) were observed in the year 2016/17 whereas, during 2017/18 also GCV and PCV recorded were high. Heritability and genetic advance as per cent mean during both the years were high. Among two years, heritability (91.38 %) was highest in 2017/18 and genetic advance as per cent mean (57.62 %) was highest during 2016/17.

Number of tillers per clump exhibited high GCV (33.57 %) as well as PCV (38.82 %) which was revealed by the pooled data analysis. Similarly, heritability (74.78 %) and GAM (59.80 %) were also high for this trait.

4.2.3.3 Number of leaves on main shoot

GCV for this trait was low (9.52 %) during 2016/17 whereas moderate (12.71) during 2017/18. PCV was moderate during both the years. Heritability was high during both the years. GAM was high (20.52 %) during 2017/18.





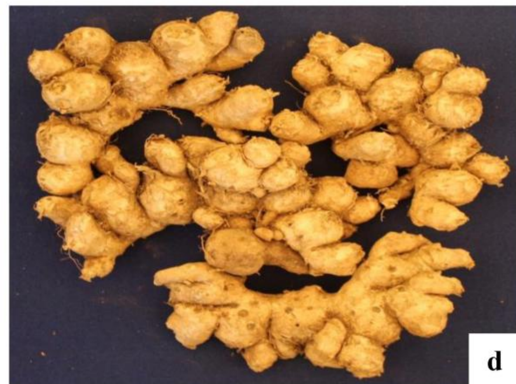


Plate 7: Rhizomes of ginger genotypes – a. Acc. 219; b. Acc. 833; c. RG 3; d. Black ginger; e. Red ginger

Table 8: Range, mean, coefficient of variation, heritability, genetic advance and genetic advance as per cent mean for different characters during 2016/17 and 2017/18

Characters	Year	Range	Mean	Genotypic coefficient of variation	Phenotypic coefficient of variation	Heritability in broad sense (%)	Genetic advance	Genetic advance as per cent mean
Plant height (cm)	2016/17	48.00-69.90	57.17	8.29	9.95	69.43	8.13	14.23
	2017/18	53.20-78.60	65.18	9.63	12.80	56.58	9.73	14.93
Number of tillers	2016/17	4.90-14.60	8.54	29.50	31.12	89.85	4.92	57.62
	2017/18	5.8-15.90	9.89	28.92	30.25	91.38	5.63	56.94
Number of leaves on main shoot	2016/17	11.90-20.60	17.59	9.52	12.28	60.04	2.67	15.19
	2017/18	8.80-15.30	10.88	12.71	16.21	61.43	2.23	20.52
Total number of leaves	2016/17	49.90-125.50	88.29	21.82	22.93	90.53	37.77	42.78
	2017/18	49.40-133.80	89.82	22.60	24.58	84.57	38.46	42.82
Leaf width (cm)	2016/17	1.59-2.45	2.01	6.73	11.39	34.88	0.16	8.18
	2017/18	1.85-2.64	2.19	8.45	10.55	64.20	0.30	13.96
Leaf area (cm^2)	2016/17	17.22-31.53	24.36	10.04	16.47	37.21	3.07	12.68
	2017/18	23.74-39.32	30.55	11.35	15.47	53.86	5.24	17.17
Shoot diameter (cm)	2016/17	2.11-2.94	2.59	5.99	10.37	33.38	0.18	7.13
	2017/18	2.15-3.41	2.83	9.35	10.73	76.02	0.47	16.80
Rhizome thickness (cm)	2016/17	1.61-2.61	2.11	8.78	10.55	69.21	0.31	15.05
	2017/18	1.57-2.40	2.12	11.96	12.35	93.76	0.51	23.86
Yield per plant (g)	2016/17	49.90-207.5	144.87	24.80	26.06	90.54	70.43	48.62
	2017/18	54.00-195.20	139.57	24.97	26.35	89.99	68.11	48.80
Projected yield (t/ha)	2016/17	4.39-18.26	12.74	24.80	26.06	90.54	6.20	48.62
	2017/18	4.75-17.17	12.28	24.97	26.32	89.99	5.99	48.80
Dry recovery (%)	2016/17	13.33-27.86	22.45	12.88	15.88	65.81	4.83	21.53
	2017/18	16.33-24.72	21.58	8.78	13.18	44.38	2.60	12.06
Essential oil (%)	2016/17	1.2-6.00	2.03	43.85	44.92	95.32	1.80	88.20
	2017/18	1.40-6.00	2.36	39.53	39.71	99.10	1.91	81.07
Oleoresin (%)	2016/17	3.29-12.18	5.60	32.88	33.22	97.95	3.75	67.03
	2017/18	2.94-12.18	5.56	33.60	33.74	99.17	3.83	68.93
Crude fibre (%)	2016/17	2.30-9.84	5.59	32.86	34.02	93.29	3.66	65.38
	2017/18	2.37-9.17	5.52	30.87	32.34	91.10	3.35	60.71

GCV & PCV: 0-10% - low; 10.1-20%-Moderate; >20.1%-High. Heritability: 0-30% - Low; 30-60%-Moderate; 60% & above-High.
GAM: 0-10% - low; 10-20%-Moderate; >20.1%-High

GCV (11.12 %), PCV (15.93 %), heritability in broad sense (48.69 %) and GAM (15.98 %) values from the pooled data analysis found to be moderate for number of leaves on main shoot.

4.2.3.4 Total number of leaves

GCV and PCV were high during both the years. Highest GCV (22.60 %) was observed during 2017/18 and PCV was also highest (24.58 %) during same year. The estimate of high heritability with high genetic advance as per cent mean was exhibited during both the years.

High GCV (23.30 %), PCV (28.94 %), heritability (64.82 %) and GAM (38.64 %) were noticed in the pooled data analysis for total number of leaves.

4.2.3.5 Leaf width

GCV was low during both the years, whereas PCV was moderate. Heritability was moderate (34.88 %) during 2016/17, whereas, it was high (64.20 %) during 2017/18. GAM was moderate during 2017/18 whereas it was low during 2017/18.

From the pooled mean analysis, it was found that GCV (7.25 %) and GAM (8.95 %) were low for leaf width, whereas PCV (12.10 %) and heritability (35.92 %) were moderate.

4.2.3.6 Leaf area

GCV (10.04 % & 11.35 % respectively) and PCV (16.47 % & 15.47 % respectively) were moderate during both the years. Whereas, heritability was low and GAM was moderate during both the years.

Low GCV (10.05 %) and moderate PCV (17.48 %), heritability (33.04 %) and GAM (11.90 %) were observed from the pooled analysis in case of leaf area of ginger.

Table 9: Pooled analysis of range, mean, coefficient of variation, heritability, genetic advance and genetic advance as per cent mean for different characters

Characters	Range	Mean	Genotypic coefficient of variation	Phenotypic coefficient of variation	Heritability in broad sense (%)	Genetic advance	Genetic advance as per cent mean
Plant height (cm)	52.65-73.01	61.09	9.12	13.47	45.84	7.77	12.72
Number of tillers	6.00-15.25	9.22	33.57	38.82	74.78	5.51	59.80
Number of leaves on main shoot	11.20-16.90	14.24	11.12	15.93	48.69	2.28	15.98
Total number of leaves	49.65-127.45	89.06	23.30	28.94	64.82	34.41	38.64
Leaf length (cm)	18.13-21.08	19.55	3.26	7.41	19.35	0.58	2.95
Leaf width (cm)	1.84-2.35	2.11	7.25	12.10	35.92	0.19	8.95
Leaf area (cm^2)	22.63-33.11	27.45	10.05	17.48	33.04	3.27	11.90
Shoot diameter (cm)	2.18-3.13	2.71	7.66	11.88	41.54	0.28	10.17
Rhizome thickness (cm)	1.59-2.55	2.12	11.56	14.11	67.19	0.41	19.52
Yield per plant (g)	51.95-201.35	142.22	32.68	34.93	87.52	89.56	62.98
Projected yield (t/ha)	4.57-17.72	12.52	32.68	34.93	87.52	7.88	62.98
Dry recovery (%)	15.32-26.14	22.02	14.68	17.96	66.82	5.44	24.72
Essential oil (%)	1.40-6.00	2.20	55.99	57.81	93.79	2.46	111.69
Oleoresin (%)	3.12-12.18	5.58	44.31	45.86	93.38	4.92	88.21
Crude fibre (%)	2.34-9.51	5.56	45.63	46.30	97.13	5.15	92.64

GCV & PCV: 0-10% - low; 10.1-20%-Moderate; >20.1%-High. Heritability: 0-30% - Low; 30-60%-Moderate; 60% & above-High.
GAM: 0-10% - low; 10-20%-Moderate; >20.1%-High

4.2.3.7 Shoot diameter

GCV for shoot diameter was low during both the years, whereas PCV was moderate. Heritability was moderate (33.38 %) during 2016/17 and high (76.02 %) during 2017/18. GAM was low during 2016/17 and moderate during 2017/18.

Low GCV (7.66 %) and moderate PCV (11.88 %), heritability (41.54 %) and GAM (10.17 %) were observed from the pooled mean analysis in case of shoot diameter of ginger.

4.2.3.8 Rhizome thickness

GCV was low during 2016/17 and moderate during 2017/18. Whereas, PCV was moderate during both the years. During 2016/17 heritability was high (69.21 %) and GAM was moderate (15.05 %). Heritability (93.76 %) and genetic advance as per cent mean (23.86 %) was highest in 2017/18.

By the analysis of pooled data, it was observed that, variability parameters such as GCV (11.56 %), PCV (14.11 %) and GAM (19.52) were found to be moderate in case of rhizome thickness, whereas, heritability was found to be high (67.19 %).

4.2.3.9 Yield per plant

GCV, PCV, heritability and GAM were high during both the years. Highest values for GCV (24.97 %), PCV (26.35 %) and GAM (48.80 %) were observed during 2017/18 in comparison to 2016/17, whereas heritability was high (90.54 %) during 2016/17.

High GCV (32.68 %), PCV (34.93 %), heritability (87.52 %) and GAM (62.98 %) were recorded for this trait, which was observed from the pooled data analysis.

4.2.3.10 Projected yield

In case of projected yield also, similar trend was observed as that of yield per plant.

4.2.3.11 Dry recovery

GCV was moderate (12.88 %) during 2016/17 and low (8.78 %) during 2017/18. PCV and heritability values were moderate during both the years. GAM was high (21.53 %) during 2016/17 whereas moderate (12.06 %) during 2017/18.

Moderate GCV (14.68 %) and PCV (17.96 %) values were observed, whereas high heritability (66.82 %) and GAM (22.72 %) were noted for dry recovery from the pooled data analysis.

4.2.4 Correlation studies

The correlation coefficients among the different characters were worked out at phenotypic (Table 10) and genotypic levels (Table 11). In general, the genotypic correlation coefficients were higher in magnitude than phenotypic correlation coefficients.

4.2.4.1 Phenotypic correlations

Phenotypic correlation coefficients showed that, yield per plant had significant and positive correlation with plant height (0.201), number of leaves on main shoot (0.293), shoot diameter (0.221), rhizome thickness (0.383) and dry recovery (0.201). Plant height showed highly significant positive correlation with the number of leaves on main shoot (0.324), shoot diameter (0.270), rhizome thickness (0.297) and significant correlation with leaf length (0.237). Number of tillers showed highly significant and positive correlation with total number

of leaves. Number of leaves on main shoot had highly significant correlation with shoot diameter (0.390). Leaf area has highly significant and positive correlation with leaf length (0.823) and leaf width (0.926).

Essential oil has shown highly significant and positive correlation with oleoresin (0.864) and crude fibre (0.433) & crude fibre exhibited highly significant positive correlation with oleoresin (0.599). Whereas, the rhizome thickness exhibited significant and negative correlation with essential oil (-0.192), oleoresin (-0.312) and crude fibre (-0.331).

Table 10: Estimates of phenotypic correlation coefficients among different traits in ginger

	Plant ht. (cm)	No. of tillers	No. of lvs on main shoot	Total No. of lvs	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)	Shoot diameter (cm)	Rhizome thickness (cm)	Yield /plant (g)	Dry recovery (%)	Essential oil (%)	Oleoresin (%)
No. of tillers	-0.237*	1											
No. of lvs on main shoot	0.324**	-0.364**	1										
Total no. of lvs	-0.055^{NS}	0.836**	0.001^{NS}	1									
Leaf length (cm)	0.237*	-0.120^{NS}	-0.025^{NS}	-0.152^{NS}	1								
Leaf width (cm)	0.045^{NS}	0.134^{NS}	-0.159^{NS}	-0.007^{NS}	0.560**	1							
Leaf area	0.139^{NS}	0.043^{NS}	-0.122^{NS}	-0.071^{NS}	0.823**	0.926**	1						
Shoot diameter (cm)	0.270**	-0.178^{NS}	0.390**	0.015^{NS}	0.167^{NS}	0.151^{NS}	0.175^{NS}	1					
Rhizome thickness (cm)	0.297**	-0.419**	0.224*	-0.330**	0.076^{NS}	0.017^{NS}	0.052^{NS}	0.288**	1				
Yield/plant (g)	0.201*	0.372**	0.293**	-0.180^{NS}	0.112^{NS}	-0.039^{NS}	0.029^{NS}	0.221*	0.383**	1			
Dry recovery (%)	0.167^{NS}	-0.201*	0.019^{NS}	-0.080^{NS}	0.033^{NS}	-0.152^{NS}	-0.101^{NS}	0.081^{NS}	0.054^{NS}	0.201*	1		
Essential oil (%)	-0.198*	0.150^{NS}	0.030^{NS}	0.030^{NS}	0.161^{NS}	0.143^{NS}	0.174^{NS}	0.028^{NS}	-0.192*	-0.396**	-0.526**	1	
Oleoresin (%)	-0.300**	0.346**	-0.123^{NS}	0.147^{NS}	0.061^{NS}	0.184^{NS}	0.156^{NS}	-0.019^{NS}	-0.312**	-0.494**	-0.605**	0.864**	1
Crude fibre (%)	-0.197*	0.201*	-0.047^{NS}	0.054^{NS}	0.024^{NS}	0.159^{NS}	0.113^{NS}	-0.190*	-0.331**	-0.253**	-0.447**	0.433**	0.599**

*Significant @ 5% level of significance; **Significant @ 1% level of significance

Table 11: Estimates of genotypic correlation coefficients among different traits in ginger

	Plant ht. (cm)	No. of tillers	No. of lvs on main shoot	Total No. of lvs	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)	Shoot diameter (cm)	Rhizome thickness (cm)	Yield /plant (g)	Dry recovery (%)	Essential oil (%)	Oleoresin (%)
No. of tillers	-0.557**	1											
No. of lvs on main shoot	0.862**	-0.553**	1										
Total no. of lvs	-0.085^{NS}	0.869**	-0.120^{NS}	1									
Leaf length (cm)	0.092^{NS}	-0.468**	0.450**	-0.538**	1								
Leaf width (cm)	-0.319**	0.200*	-0.070^{NS}	-0.022^{NS}	0.742**	1							
Leaf area	-0.188*	-0.028^{NS}	0.114^{NS}	-0.207*	0.908**	0.956**	1						
Shoot diameter (cm)	0.390**	-0.290**	0.877**	-0.003^{NS}	0.558**	0.095^{NS}	0.281**	1					
Rhizome thickness (cm)	0.289**	-0.546**	0.335**	-0.362**	0.377**	0.331**	0.378**	0.748**	1				
Yield /plant (g)	0.408**	0.443**	0.403**	-0.267**	0.206*	0.030^{NS}	0.140^{NS}	0.353**	0.589**	1			
Dry recovery (%)	0.526**	-0.329**	0.166^{NS}	-0.124^{NS}	0.034^{NS}	-0.655**	-0.455**	0.135^{NS}	0.208*	0.276**	1		
Essential oil (%)	-0.365**	0.145^{NS}	0.089^{NS}	0.030^{NS}	0.672**	0.427**	0.531**	0.259**	-0.289**	-0.444**	-0.715**	1	
Oleoresin (%)	-0.566**	0.378**	-0.209*	0.153^{NS}	0.323**	0.420**	0.410**	-0.111^{NS}	-0.421**	-0.561**	-0.885**	0.915**	1
Crude fibre (%)	-0.401**	0.277**	-0.029^{NS}	0.112^{NS}	-0.037^{NS}	0.283**	0.180^{NS}	-0.339**	-0.482**	-0.301**	-0.632**	0.478**	0.662**

*Significant @ 5% level of significance; **Significant @ 1% level of significance

4.2.4.2 Genotypic correlations

The genotypic correlation coefficients between different characters revealed that yield per plant had highly significant and positive correlation with plant height (0.408), number of leaves on main shoot (0.403), shoot diameter (0.353), rhizome thickness (0.589), dry recovery (0.276) and leaf length (0.206). Similarly like phenotypic correlation, genotypic correlation for plant height showed positive association with number of leaves on main shoot (0.862), shoot diameter (0.390) and rhizome thickness (0.289). Number of tillers had highly significant positive correlation with total number of leaves (0.869). Number of leaves on main shoot exhibited positive correlation with leaf length (0.450), shoot diameter (0.877) and rhizome thickness (0.335).

Leaf area had highly significant and positive correlation with leaf length (0.908) and leaf width (0.956). Leaf length showed positive association with leaf width (0.742), shoot diameter (0.558), rhizome thickness (0.337), essential oil (0.672) and oleoresin (0.323).

Essential oil showed highly significant and positive correlation with oleoresin (0.915) and crude fibre (0.478). Crude fibre had highly significant and positive correlation with oleoresin (0.662). Genotypic correlation also exhibited high negative correlation of rhizome thickness with essential oil (-0.289), oleoresin (-0.421) and crude fibre (-0.482).

4.2.5 Grouping of genotypes as per DUS guidelines

Based on the observations recorded, genotypes were grouped as per the DUS guidelines. Ginger genotypes were grouped as per the PPVFRA DUS guidelines of ginger. The two related genera genotypes such as mango ginger and black ginger is grouped as per DUS guidelines of turmeric. Results are presented in the Table 12.

4.2.5.1 Growth habit

Among thirty genotypes, sixteen genotypes (53.33 %) exhibited erect growth habit and twelve genotypes (40.00 %) exhibited semi erect growth habit as per the DUS guidelines of ginger. Other two genotypes, mango ginger and black ginger exhibited open type of growth habit (6.66 %) as per the DUS guidelines of turmeric.

4.2.5.2 Plant height

Out of thirty genotypes, twenty eight genotypes (93.33 %) were short as per the ginger guidelines. Black ginger was short (3.33 %) and mango ginger was tall (3.33 %) as per the DUS guidelines of turmeric.

4.2.5.3 Number of tillers

Number of tillers was grouped as few, medium and many. Nineteen genotypes (63.33 %) had few tillers, eight genotypes (26.66 %) had medium number of tillers and one genotype *i.e.,* Mahim had many tillers (3.33 %) as per ginger guidelines. As per the DUS guidelines of turmeric, mango ginger had few tillers (3.33 %) and black ginger had many tillers (3.33 %).

4.2.5.4 Shoot diameter

Classification of genotypes based on shoot diameter is available only in ginger DUS guidelines and not available in turmeric. So, only twenty eight ginger genotypes were classified as per this trait. Based on shoot diameter, ginger genotypes classification are narrow, medium and broad. Out of twenty eight genotypes, twenty seven genotypes (96.43 %) had narrow shoot diameter and only one genotype Aswathy had medium (3.57 %) shoot diameter.

4.2.5.5 Number of leaves on main shoot

Among thirty genotypes, twenty eight ginger genotypes (93.33 %) exhibited few number of leaves on main shoot as per ginger DUS guidelines and as per turmeric DUS black ginger (3.33 %) exhibited few number of leaves on main shoot, whereas mango ginger (3.33 %) exhibited intermediate number of leaves on main shoot.

Table 12: Grouping of ginger genotypes as per the DUS guidelines (PPVFRA, 2007)

Characteristic	Status	Note	Number of genotypes	Genotypes
Growth habit	Erect	1	16	Suravi, Suruchi, Aswathy, KAU Chandra, Nadia, Maran, Gorubathane, Mahim, Zaheerabad local, Andhra Pradesh local, Acc. 247, Acc. 65, Acc. 578, Acc. 219, RG 3, Red ginger
	Semi erect	3	12	IISR Varada, IISR Mahima, IISR Rejatha, Suprabha, Athira, Karthika, Sourabh, Mohini, Rio de Janeiro, Himachal, Bhaise, Acc. 833
	Spreading	5		Nil
	Compact	1		Nil
	Open	9	2	Black ginger, Mango ginger
Plant height	Short (<100)	3	28	IISR Varada, IISR Mahima, IISR Rejatha, Suprabha, Suravi, Suruchi, Athira, Karthika, Aswathy, Sourabh, Mohini, KAU Chandra, Rio de Janeiro, Nadi, Maran, Himachal, Bhaise, Gorubathane, Mahim, Zaheerabad local, Arunachal Pradesh local, Acc. 247, Acc. 65, Acc. 578, Acc. 219, Acc. 833, RG 3, Red ginger
	Medium (100-120)	5		Nil
	Tall (>120)	7		Nil
	Short (<85)	3	1	Black ginger
	Medium (85-100)	5		Nil
	Tall (>100)	7	1	Mango ginger
Number of tillers	Few (<10)	3	19	IISR Varada, IISR Mahima, IISR Rejatha, Athira, Karthika, Mohini, KAU Chandra, Nadia, Maran, Himachal, Bhaise, Gorubathane, Zaheerabad local, Acc. 247, Acc. 65, Acc. 578, Acc. 219, Acc. 833, Red ginger
	Medium (10-15)	5	8	Suprabha, Suravi, Suruchi, Aswathy, Sourabh, Rio de Janeiro, Arunachal Pradesh local, RG 3
	Many (>15)	7	1	Mahim
	Few (<3)	1	1	Mango ginger
	Medium (3-5)	3		
	Many (>5)	5	1	Black ginger

Characteristic	Status	Note	Number of genotypes	Genotypes
Shoot diameter	Narrow (<3)	3	27	IISR Varada, IISR Mahima, IISR Rejatha, Suprabha, Suravi, Suruchi, Athira, Karthika, Sourabh, Mohini, KAU Chandra, Rio de Janeiro, Nadia, Maran, Himachal, Bhaise, Gorubathane, Mahim, Zaheerabad local, Arunachal Pradesh local, Acc. 247, Acc. 65, Acc. 578, Acc. 219, Acc. 833, RG 3, Red ginger
	Medium (3-5)	5	1	Aswathy
	Broad (>5)	7		Nil
Number of leaves on main shoot	Few (<25)	3	28	IISR Varada, IISR Mahima, IISR Rejatha, Suprabha, Suravi, Suruchi, Athira, Karthika, Sourabh, Mohini, KAU Chandra, Rio de Janeiro, Nadia, Maran, Himachal, Bhaise, Gorubathane, Mahim, Zaheerabad local, Arunachal Pradesh local, Acc. 247, Acc. 65, Acc. 578, Acc. 219, Acc. 833, RG 3, Red ginger
	Medium (25-35)	5		Nil
	Many (>35)	7		Nil
	Few (<5)	3	1	Black ginger
	Intermediate (5-10)	5	1	Mango ginger
	Many (>10)	7		Nil
Leaf length	Short (<25)	3	28	IISR Varada, IISR Mahima, IISR Rejatha, Suprabha, Suravi, Suruchi, Athira, Karthika, Sourabh, Mohini, KAU Chandra, Rio de Janeiro, Nadia, Maran, Himachal, Bhaise, Gorubathane, Mahim, Zaheerabad local, Arunachal Pradesh local, Acc. 247, Acc. 65, Acc. 578, Acc. 219, Acc. 833, RG 3, Red ginger
	Medium (25-30)	5		Nil
	Long (>30)	7		Nil
	Short (<30)	3	1	Black ginger
	Medium (30-40)	5		Nil
	Long (>40)	7	1	Mango ginger

Characteristic	Status	Note	Number of genotypes	Genotypes
Leaf width	Narrow (<2.5)	3	28	IISR Varada, IISR Mahima, IISR Rejatha, Suprabha, Suravi, Suruchi, Athira, Karthika, Sourabh, Mohini, KAU Chandra, Rio de Janeiro, Nadia, Maran, Himachal, Bhaise, Gorubathane, Mahim, Zaheerabad local, Arunachal Pradesh local, Acc. 247, Acc. 65, Acc. 578, Acc. 219, Acc. 833, RG 3, Red ginger
	Medium (2.5-3.5)	5		Nil
	Broad (>3.5)	7		Nil
	Narrow (<10)	3		Nil
	Medium (10-15)	5	2	Black ginger, Mango ginger
	Broad (>15)	7		Nil
Rhizome thickness	Thin (<2)	3	4	Karthika, Rio de Janeiro, Mahim, Arunachal Pradesh local
	Medium (2-3)	5	24	IISR Varada, IISR Mahima, IISR Rejatha, Suprabha, Suravi, Suruchi, Athira, Sourabh, Mohini, KAU Chandra, Nadia, Maran, Himachal, Bhaise, Gorubathane, Mahim, Zaheerabad local, Acc. 247, Acc. 65, Acc. 578, Acc. 219, Acc. 833, RG 3, Red ginger
	Bold (>3)	7		Nil
Rhizome shape	Straight	1	9	IISR Mahima, Aswathy, KAU Chandra, Maran, Arunachal Pradesh local, Acc. 247, Acc. 219, Acc. 833, RG 3
	Curved	3	9	Suravi, Suruchi, Karthika, Sourabh, Mohini, Rio de Janeiro, Nadia, Himachal, Gorubathane
	Zigzagged	5	10	IISR Varada, IISR Rejatha, Suprabha, Athira, Bhaise, Mahim, Zaheerabad local, Acc. 65, Acc. 578, Red ginger
	Straight	3	1	Mango ginger
	Curved	5	1	Black ginger
Dry recovery	Low (<16)	3	1	Red ginger
	Medium (16-18)	5	1	Rio de Janeiro
	High (>18)	7	26	IISR Varada, IISR Rejatha, IISR Mahima, Suprabha, Suravi, Suruchi, Athira, Karthika, Aswathy, Sourabh, Mohini, KAU Chandra, Nadia, Maran, Himachal, Bhaise, Gorubathane, Mahim, Zaheerabad local, Arunachal Pradesh local, Acc. 247, Acc. 65, Acc. 578, Acc. 219, Acc. 833, RG 3

Characteristic	Status	Note	Number of genotypes	Genotypes
	Low (<15)	3		Nil
	Intermediate (15-20)	5		Nil
	High (>20)	7	2	Black ginger, Mango ginger

**Grouping of ginger genotypes is as per DUS guidelines of ginger and that of mango ginger and black ginger is as per the DUS guidelines of turmeric*

4.2.5.6 Leaf length

All the twenty eight ginger genotypes (93.33 %) showed short leaf length as per ginger DUS guidelines whereas black ginger (3.33 %) also had short leaf length and mango ginger (3.33 %) had long leaf length as per turmeric DUS guidelines

4.2.5.7 Leaf width

In case of leaf width also, twenty eight ginger genotypes (93.33 %) exhibited narrow leaf width, whereas two genotypes *viz.,* black ginger and mango ginger exhibited medium leaf width (6.66 %).

4.2.5.8 Rhizome thickness

Grouping of genotypes with regard to the rhizome thickness is available only in ginger DUS guidelines and not available in case of turmeric. As per this, four genotypes (14.28 %) showed thin rhizome thickness and twenty four genotypes (85.72 %) exhibited medium rhizome thickness

4.2.5.9 Rhizome shape

Genotypes were grouped as straight, curved or zigzagged as per the DUS guidelines. As per the ginger guidelines nine genotypes (30.00 %) had straight rhizome, nine genotypes (30.00 %) showed curved shaped rhizomes and ten genotypes (33.33 %) manifested zigzagged rhizome shape. As per turmeric guidelines, mango ginger (3.33 %) had straight rhizome and black ginger (3.33 %) had curved rhizome.

4.2.5.10 Dry recovery

As per the dry recovery, genotypes were grouped into genotypes with high, medium and low dry recovery. Red ginger (3.33 %) exhibited low dry recovery, Rio de Janeiro (3.33 %) showed medium dry recovery and other twenty six ginger genotypes (86.66 %) exhibited high dry recovery of > 18% as per the ginger guidelines. As per the turmeric guidelines, both black ginger and mango ginger exhibited high dry recovery (6.66 %) of >20 %.

4.3 Development of chemical profiles

4.3.1 Quality characters

Biochemical parameters of 28 ginger genotypes with respect to essential oil, oleoresin and crude fibre are presented in table 13.

4.3.1.1 Essential oil

Significant differences were observed among the genotypes for essential oil content during both the growing seasons.

During 2016/17 highest essential oil content of 6.0 % was recorded in red ginger and lowest was in KAU Chandra (1.2 %).

In 2017/18 also same trend was observed for highest essential oil content *i.e.,* in red ginger (6.0 %). Lowest oil content of 1.4 % was recorded in Bhaise, Gorubathane and Acc. 833

In the pooled mean analysis also, same trend was observed for highest oil content *i.e.,* red ginger (6.0 %). Lowest oil content was 1.40 % in KAU Chandra, Gorubathane and Acc. 833.

4.3.1.2 Oleoresin

Oleoresin percentage showed highly significant differences among the genotypes.

Higher oleoresin percentage of 12.18 % was recorded in red ginger and lowest was in Acc. 578 (3.28 %) during 2016/17. During the second year of experimentation also same trend was prevailed where higher oleoresin percentage of 12.18 % was obtained in red ginger and lowest was 2.94 % in Acc. 578.

Pooled mean analysis of two years data showed that, red ginger had the highest oleoresin percentage of 12.18 % and lowest was in Acc. 578 (3.11 %).

Table 13: Biochemical parameters of ginger genotypes

Variety/Genotype	Essential oil (%)			Oleoresin (%)			Crude fibre (%)			Essential oil yield per unit area (l/ha)
	2016/17	2017/18	Pooled	2016/17	2017/18	Pooled	2016/17	2017/18	Pooled	
IISR Varada	1.6	1.7	1.65	4.10	3.63	3.87	5.07	5.05	5.07	59.36
IISR Mahima	1.4	2.1	1.75	4.45	4.76	4.60	4.84	4.95	4.90	34.57
IISR Rejatha	1.6	1.8	1.70	5.02	3.83	4.43	6.08	6.05	6.07	51.71
Suprabha	1.4	2.4	1.90	4.62	6.19	5.40	6.31	6.33	6.32	39.03
Suravi	1.8	2.3	2.05	5.44	5.78	5.61	3.85	3.95	3.90	46.93
Suruchi	1.9	3.4	2.65	5.87	7.24	6.55	5.34	5.20	5.27	69.91
Athira	2.2	2.0	2.10	5.27	5.53	5.40	3.80	3.95	3.88	64.50
Karthika	2.0	2.1	2.05	5.35	4.46	4.91	4.99	5.25	5.12	76.17
Aswathy	2.6	3.0	2.80	6.28	6.73	6.50	3.58	3.68	3.64	82.45
Sourabh	2.2	2.1	2.15	4.78	4.23	4.51	4.35	4.42	4.39	55.90
Mohini	1.4	2.4	1.90	3.78	6.30	5.04	6.36	6.40	6.38	50.54
KAU Chandra	1.2	1.6	1.40	4.43	3.83	4.13	4.03	4.15	4.09	47.18
Rio de Janeiro	2.6	2.6	2.60	8.57	6.98	7.77	6.21	6.11	6.16	44.88
Nadia	1.6	1.8	1.70	3.64	4.20	3.92	2.90	2.82	2.86	44.02
Maran	2.4	3.5	2.95	5.33	4.82	5.07	4.21	4.21	4.21	106.94
Himachal	1.6	1.8	1.70	4.6	4.34	4.47	5.15	5.03	5.09	64.50
Bhaise	2.0	1.4	1.70	5.22	5.74	5.48	4.46	4.51	4.48	48.64
Gorubathane	1.4	1.4	1.40	4.03	4.25	4.14	4.63	4.53	4.58	41.59
Mahim	1.8	3.0	2.40	6.52	6.92	6.72	6.70	6.50	6.60	57.70
Zaheerabad local	2.0	2.0	2.00	5.79	3.56	4.68	5.63	5.58	5.61	61.66
Arunachal Pradesh local	3.0	3.0	3.00	8.55	8.55	8.55	8.61	8.71	8.66	26.11
Acc. 247	2.4	1.9	2.15	7.01	4.51	5.76	5.77	5.57	5.67	73.20
Acc. 65	2.6	3.0	2.80	7.42	6.79	7.10	7.77	7.37	7.57	70.45
Acc. 578	1.4	1.6	1.50	3.28	2.94	3.11	2.30	2.37	2.34	45.71
Acc. 219	1.6	2.0	1.80	4.45	5.53	4.99	9.28	9.17	9.23	64.17
Acc. 833	1.4	1.4	1.40	5.63	5.55	5.59	8.39	7.99	8.19	38.25
RG 3	2.0	2.8	2.40	5.04	6.33	5.69	6.27	6.01	6.14	73.53
Red ginger	6.0	6.0	6.00	12.18	12.18	12.18	9.85	9.17	9.51	58.53
Mean	**2.04**	**2.36**	**2.20**	**5.60**	**5.56**	**5.58**	**5.59**	**5.52**	**5.56**	**57.08**
CD @ 5%	**0.40**	**0.18**	**0.44**	**0.54**	**0.35**	**0.92**	**1.01**	**1.09**	**0.81**	**16.16**
CV (%)	**9.71**	**3.76**	**14.40**	**4.75**	**3.07**	**11.79**	**8.81**	**9.64**	**7.85**	**20.12**

4.3.1.3 Crude fibre

Significant differences were observed in respect of crude fibre among different ginger genotypes during two experimental seasons.

During 2016/17 lowest crude fibre of 2.30 % was observed in Acc. 578 and highest was in red ginger (9.85 %). The genotype Nadia (2.90 %) was found to be at par with Acc. 578.

In 2017/18 also, fibre content was lowest in Acc. 578 (2.37 %) and highest in red ginger (9.17 %) and Acc. 219 (9.17 %). Genotype Nadia with fibre content of 2.82 % was at par with Acc. 578.

Pooled mean analysis also exhibited that, Acc. 578 was low in fibre (2.34 %) and red ginger had high fibre content of 9.51 %. Nadia (2.86) was at par with Acc. 578.

4.3.1.4 Essential oil yield per unit area (l/ha)

Highest essential oil yield per hectare of 106.94 l ha^{-1} was obtained in the genotype Maran which was followed by the variety Aswathy (82.44 l ha^{-1}) and the lowest oil yield per unit area (26.11 l ha^{-1}) was observed in Arunachal Pradesh local. In case of red ginger which was having highest percentage of essential oil (6 %) exhibited essential oil yield per unit area of 58.53 l ha^{-1}.

4.3.2 Components of variability for quality characters

The estimates of range, general mean, coefficient of variation, heritability, genetic advance and genetic advance as per cent mean in respect of two years *viz.,* 2016/17 and 2017/18 for different quality characters are tabulated in Table 8 & 9

4.3.2.1 Essential oil

High GCV, PCV, heritability coupled with GAM were observed during both the years. Highest values for GCV (43.85 %), PCV (44.92 %) and GAM (88.20 %) were observed during 2016/17, whereas highest value for heritability (99.10 %) was observed during 2017/18.

Similar to year wise results, the pooled analysis also recorded high values for GCV (55.99 %), PCV (57.81 %), heritability (93.79 %) and GAM (111.69 %) for this trait.

4.3.2.2 Oleoresin

During both the years high GCV, PCV, heritability and GAM were observed. Highest GCV of 33.60 %, PCV of 33.74 %, heritability of 99.17 % and GAM of 68.93 % were observed in the year 2017/18.

Pooled data analysis exhibited high values for GCV (44.31 %), PCV (45.86 %), heritability (93.38 %) and GAM (88.21 %).

4.3.2.3 Crude fibre

The variability parameters *viz.,* GCV (32.86 %), PCV (34.02 %), heritability (93.29 %) and GAM (65.38 %) were found to be highest during 2016/17 as compared to 2017/18.

Crude fibre also recorded high GCV (45.63 %), PCV (46.30 %), heritability (97.13 %) and GAM (92.64 %) values from the pooled mean analysis.

4.3.3 GC/MS analysis of ginger essential oil

Along with 28 ginger genotypes, essential oil of mango ginger (*Curcuma amada*) and black ginger (*Kaempferia parviflora*) were also extracted, where the oil yield was 1.0 % and 0.1 % respectively. The chemical constituents identified in the essential oil of thirty genotypes detected by GC/MS analysis are presented in tables 14a, 14b and 14c and the chromatogram of GC/MS analyzed ginger genotypes is given in Fig.2. Thirty five essential oil components were identified. More number of compounds was observed in Aswathy, Suprabha and Mohini around 28 compounds and less number of compounds (13) in black ginger.

Zingiberene was the major component present in the essential oil of ginger genotypes, whereas in mango ginger it was present in less quantity (3.24 %) and absent in black ginger. Zingiberene content in ginger genotypes varied from 20.18 % (Himachal) to 33.42 % (Maran).

Beta sesquiphellandrene content in the ginger genotypes varied from 8.48 % (Acc. 833) to 21.78 % (red ginger). The percentage of this compound was less in mango ginger *i.e.,* 2.7 % whereas it was absent in black ginger.

The content of Beta bisabolene ranged from 14.09 % (IISR Varada) to 2.34 % (Sourabh). In mango ginger percentage of this compound was 0.9% and it was absent in black ginger.

The content of 1, 8-cineole ranged from 0.46 % (black ginger) to 5.37 % (Mahim). It was absent in Gorubathane.

In case of alpha farnesene, IISR Rejatha had higher content of 15.31 % and it was lowest in mango ginger (0.81 %). This compound was not present in black ginger.

Alpha pinene content varied from 0.25 % (Acc. 833) to 13.17 % (black ginger). In black ginger it was found to be one of the major compounds. Maximum quantity of camphene was observed in black ginger (20.21 %) and minimum was in Acc. 833 (0.9 %). Camphene was also found to be one of the major compounds in black ginger.

In mango ginger, the quantity of beta pinene was maximum (15.17 %) and in black ginger it was 2.0 %. In case of ginger genotypes it was in very less quantity and in some genotypes it was absent.

Beta myrcene content varied from 0.35 % (Acc. 833) to 1.06 % (red ginger). In many of the ginger genotypes, it was in less quantity and it was absent in Mango ginger, ginger genotypes *viz.,* Karthika, Mahim, Suprabha, RG 3 and Suruchi.

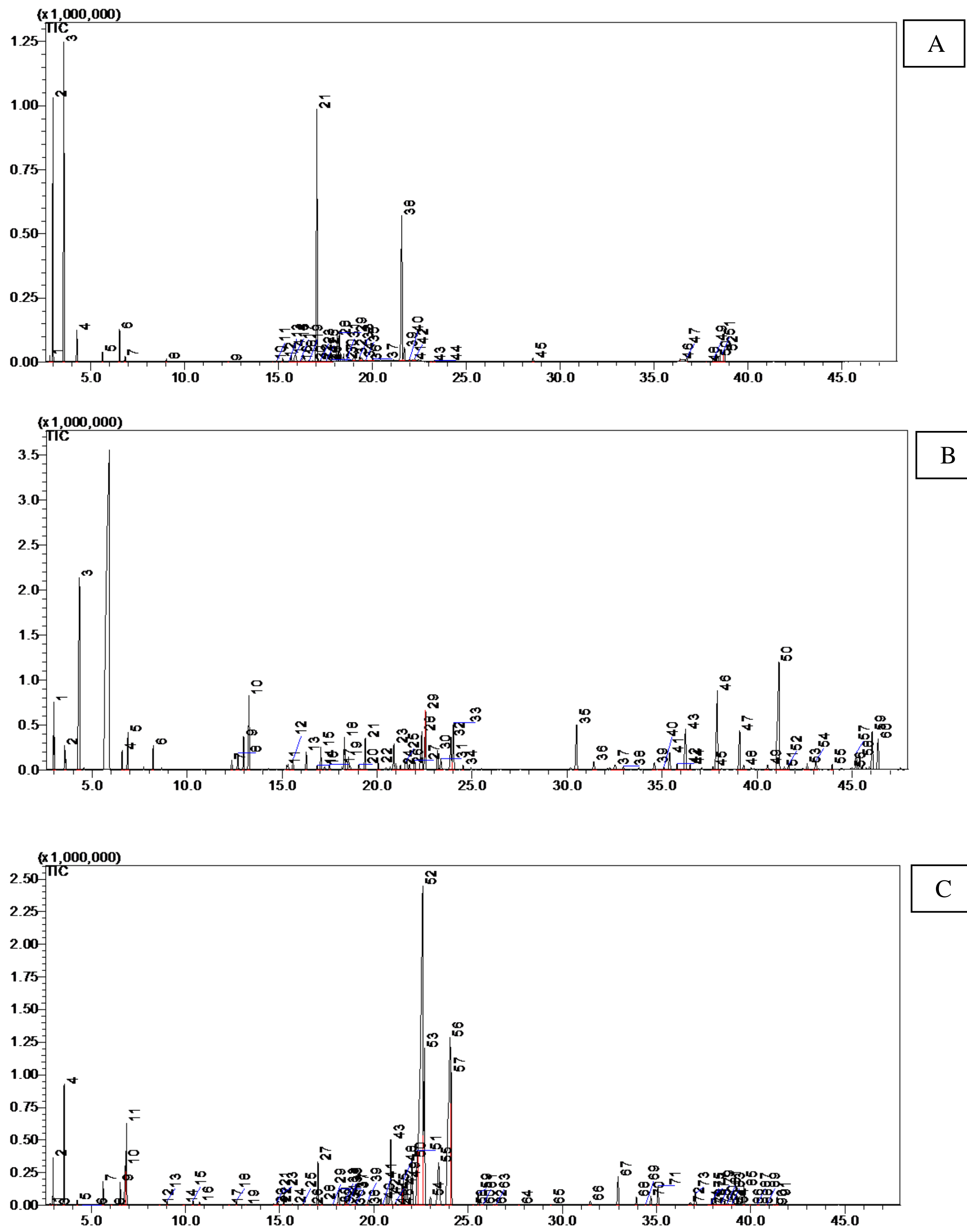


Fig. 2: Chromatogram of GCMS analyzed ginger genotypes. A. Black ginger, B. Mango ginger, C. Ginger *cv*. Maran

Table 14a: Chemical composition of essential oil from thirty ginger genotypes

Compound (%)	AI	KI	Varada	Mahima	Rejatha	Suprabha	Suravi	Suruchi	Athira	Karthika	Aswathy	Sourabh
Alpha.-Pinene	932	939	0.66	0.76	0.73	0.71	0.68	1.19	0.98	0.38	1.04	0.46
Camphene	946	954	2.46	2.41	2.63	2.54	2.36	3.32	3.22	1.45	3.43	1.77
Beta.-Pinene	974	979	0.04	0.1	0.06	0.13	-	0.26	0.15	-	0.18	-
Beta.-Myrcene	988	990	0.68	0.89	0.77	-	0.82	-	0.71	-	0.93	0.69
Alpha-Limonene	1024	1029	0.58	0.75	0.69	0.64	0.80	1.04	0.81	0.42	0.99	0.63
Beta-Phellandrene	1025	1029	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace
1,8 cineole	1026	1031	3.44	3.73	3.73	3.78	4.70	4.43	4.64	2.69	5.06	3.44
beta- (2) Ocimene	1044	1050	-	-	-	-	-	-	-	-	-	-
Terpinolene	1086	1088	0.10	0.17	0.11	0.12	0.14	0.23	0.14	0.08	0.21	0.13
Furan	1031	1037	-	-	-	-	-	-	-	-	-	-
Alpha.-Copaene	1374	1376	0.29	0.48	-	0.40	0.50	0.48	0.72	0.48	0.49	0.37
beta.-Linalool	1095	1096	0.84	0.94	1.14	0.78	1.66	0.99	0.85	0.43	1.8	0.82
Beta-Elemene	1389	1390	0.42	0.86	0.59	0.74	1.05	1.05	0.70	0.80	0.85	0.65
Alloaromadendrene	1458	1460	-	0.22	-	0.29	0.38	0.53	0.39	0.29	-	0.2
beta.-Farnesene	1440	1442	0.32	-	0.47	0.51	0.72	-	0.67	0.65	0.63	0.49
beta.-Citral	1316	1318	6.62	6.63	9.51	3.95	7.01	5.77	4.12	5.79	4.91	4.61
alpha.-Terpineol	1186	1188	-	-	-	1.09	1.58	2.53	-	-	1.60	-
Borneol	1165	1169	1.95	2.55	2.63	1.05	1.25		2.62	2.83	1.26	2.09
Germacrene D	1484	1485	1.07	1.57	1.47	1.72	2.03	2.06	2.25	2.29	2.01	1.51
Gamma.-Cadinene	1513	1513	1.73	-	2.49	3.16	3.56	2.84	3.65	3.55	3.24	2.39
Gamma-Muurolene	1478	1479	-	2.29	-	-	-	-	-	-	-	-
Alpha.-Eudesma	1489	1489	-	-	-	2.12	-	-	-	-	1.93	1.77
Zingiberene	1493	1493	27.85	21.90	25.78	30.16	22.70	26.13	27.42	28.45	24.30	22.62
Beta-Bisabolene	1505	1505	14.09	4.63	6.28	6.58	3.40	4.14	4.14	6.40	5.26	2.34
alpha.-Farnesene	1505	1505	10.52	13.61	15.31	7.30	6.49	6.22	9.47	10.7	4.43	9.94
Alpha-Curcumene	1479	1480	-	-	-	-	-	-	-	-	-	-
Beta.-Sesquiphellandrene	1521	1522	17.58	16.10	13.31	19.39	18.38	16.53	16.94	19.17	20.9	17.83
Germacrene B	1559	1561		0.60	0.48	0.53	0.64	0.84	0.61	0.68	0.61	0.49
Beta.-Geraniol	1249	1242	1.22	0.90	1.36	0.37	0.42	0.49			0.09	0.75
Beta.-Caryophyllene Epoxide	1582	1583	-	-	-	-	-	-	-	-	-	-
trans-Nerolidol	1561	1563	1.46	1.90	1.88	1.33	2.31	1.50	1.57	1.40	1.75	1.53
Elemol	1548	1549	-	-	-	0.47	0.60	0.67	0.51	0.58	0.55	0.40
beta.-Eudesmol	1649	1650	0.33	1.17	0.52	0.78	1.03	1.69	0.87	0.84	0.89	0.67
Farnesal	1740	1741	-	-	-	0.12	0.19	0.68	0.30	0.25	0.19	0.14
Unknown			5.75	14.84	8.06	9.24	14.6	14.39	11.55	9.40	10.47	21.27

Table 14b: Chemical composition of essential oil from thirty ginger genotypes

Compound (%)	AI	KI	Mohini	KAU Chandra	RDJ	Nadia	Maran	Himachal	Bhaise	Gorubathane	Mahim	Zaheerabad local
Alpha.-Pinene	932	939	0.78	0.49	0.84	0.61	0.89	0.80	0.70	0.37	1.29	0.94
Camphene	946	954	2.70	1.60	2.60	2.12	3.08	2.52	2.20	1.30	3.87	3.34
Beta.-Pinene	974	979	0.15	-	0.15	-	-	-	0.15	0.47	0.22	
Beta.-Myrcene	988	990	0.81	0.63	0.72	0.68	0.72	0.95	0.73	0.45		1.03
Alpha-Limonene	1024	1029	0.80	0.58	0.79	0.60	0.73	0.85	0.64	2.37	1.16	0.87
Beta-Phellandrene	1025	1029	Trace	Trace	Trace	Trace	1.48	Trace	Trace	Trace	Trace	Trace
1,8 cineole	1026	1031	4.80	2.88	4.01	3.16	2.61	3.95	4.18	-	5.37	4.40
beta- (2) Ocimene	1044	1050	-	-	-	-	-	-	-	-	-	-
Terpinolene	1086	1088	0.16	0.14	0.17		0.12	0.19		0.10	0.21	0.15
Furan	1031	1037	-	-	-	-	-	-	-	-	-	-
Alpha.-Copaene	1374	1376	0.52	0.43	0.52	0.42	0.39	0.49	0.47	0.51	0.47	0.41
beta.-Linalool	1095	1096	1.60	0.97	1.45	0.75	1.46	1.03	0.76	0.99	0.81	1.13
Beta-Elemene	1389	1390	0.89	0.75	0.97	0.70	0.62	0.76	0.79	0.83	0.85	0.73
Alloaromadendrene	1458	1460	-	0.33		0.20	-	0.22	0.3	-	0.37	-
beta.-Farnesene	1440	1442	0.59	-	-	0.55	0.51	-	0.54	-	-	0.60
beta.-Citral	1316	1318	6.21	5.99	4.34	5.99	2.98	5.98	6.11	6.46	5.77	9.69
alpha.-Terpineol	1186	1188	1.54	-	2.52	-	0.88	-	1.34	-	1.82	
Borneol	1165	1169	1.15	2.45		2.30	1.04	2.82	0.93	2.59	1.21	2.38
Germacrene D	1484	1485	1.94	1.59	2.01	1.45		1.52	1.77	1.55	1.53	1.48
Gamma.-Cadinene	1513	1513	3.36	2.51	3.44	-	2.90	-	3.06	2.79	3.02	2.34
Gamma-Muurolene	1478	1479	-	-	-	2.26	-	2.32	-	-	-	-
Alpha.-Eudesma	1489	1489	1.87	1.33	1.60	-	-	1.47	-	1.71	1.69	-
Zingiberene	1493	1493	26.55	25.07	28.16	25.84	33.42	20.18	20.75	24.59	24.18	28.98
Beta-Bisabolene	1505	1505	5.36	5.41	5.35	4.24	7.41	4.68	3.57	5.27	5.63	3.42
alpha.-Farnesene	1505	1505	6.00	13.33	4.28	13.88	3.56	13.18	12.40	13.70	8.22	10.98
Alpha-Curcumene	1479	1480	-	-	-	-	4.09	-	-	-	-	-
Beta.-Sesquiphellandrene	1521	1522	16.60	16.18	18.60	18.60	16.91	16.72	18.99	14.96	16.20	14.51
Germacrene B	1559	1561	0.66	0.65	0.65	0.58	0.48	0.63	0.60	0.65	0.58	0.42
Beta.-Geraniol	1249	1242	0.36	1.19		0.73	-	0.96	-	1.30	0.27	1.28
Beta.-Caryophyllene Epoxide	1582	1583	-	-	-	-	-	-	-	-	-	-
trans-Nerolidol	1561	1563	1.92	2.11	1.86	1.79	1.26	1.82	1.52	2.33	1.47	1.80
Elemol	1548	1549	0.57	0.50	0.66		0.33		0.62	0.57		0.38
beta.-Eudesmol	1649	1650	0.92	1.07	1.12	0.77	-	0.91	0.89	0.94	0.93	0.51
Farnesal	1740	1741	0.26	-	-	0.19	-	0.18	-	0.26	0.20	-
Unknown			10.93	11.82	13.19	18.76	13.61	14.87	15.99	12.94	12.66	8.23

Table 14c: Chemical composition of essential oil from thirty ginger genotypes

Compound (%)	AI	KI	Arunachal local	Acc. 247	Acc. 65	Acc. 578	Acc. 219	Acc. 833	RG 3	Red ginger	Black ginger	Mango ginger
Alpha.-Pinene	932	939	1.18	0.61	1.25	0.67	0.57	0.25	0.88	1.32	13.17	2.98
Camphene	946	954	3.95	2.07	3.83	2.03	1.88	0.90	2.60	4.86	20.21	1.24
Beta.-Pinene	974	979	0.37	0.10	0.21	0.10	0.10	-	0.18	0.19	2.00	15.17
Beta.-Myrcene	988	990	0.68	0.66	1.04	0.81	0.68	0.35	-	1.06	0.68	-
Alpha-Limonene	1024	1029	0.92	0.66	1.05	0.68	0.64	0.41	0.86	1.16	2.26	0.85
Beta-Phellandrene	1025	1029	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace
1,8 cineole	1026	1031	4.70	3.31	5.27	3.59	3.24	3.12	4.26	3.51	0.46	1.93
beta- (2) Ocimene	1044	1050	-	-	-	-	-	-	-	-	-	1.13
Terpinolene	1086	1088	0.16	0.14	0.21	0.15	0.15	-	0.19	0.22	0.18	-
Furan	1031	1037	-	-	-	-	-	-	-	-	-	4.41
Alpha.-Copaene	1374	1376	0.46	0.45	0.5	0.44	0.44	0.75	0.57	0.31	0.29	-
beta.-Linalool	1095	1096	0.84	0.96	1.71	0.90	1.17	0.66	1.26	0.54	23.30	1.17
Beta-Elemene	1389	1390	0.90	0.80	0.88	0.76	0.87	1.59	1.15	0.96	2.00	-
Alloaromadendrene	1458	1460	0.32	-	0.39	0.28	-	0.48	0.51	0.14	2.34	-
beta.-Farnesene	1440	1442	0.52	0.54	-	-	-	0.51	-	0.52	-	-
beta.-Citral	1316	1318	4.23	4.88	6.48	5.87	5.77	0.97	6.51	1.75	-	1.99
alpha.-Terpineol	1186	1188	1.95	-	1.54	-	2.34	2.27	2.43	-	-	1.22
Borneol	1165	1169	1.81	2.30	1.05	2.15	-	-	-	2.99	-	-
Germacrene D	1484	1485	1.92	1.71	1.88	1.67	1.71	1.56	1.94	0.48	16.00	-
Gamma.-Cadinene	1513	1513	-	-	-	2.71	-	2.97	2.92	1.61	1.43	0.72
Gamma-Muurolene	1478	1479	2.67	2.69	3.14	-	2.69	-	-	-	-	-
Alpha.-Eudesma	1489	1489	-	1.45	1.68	1.47	-	3.13	-	-	-	-
Zingiberene	1493	1493	26.05	29.83	27.68	22.09	26.93	24.92	24.95	27.48	-	3.24
Beta-Bisabolene	1505	1505	5.54	5.91	2.52	3.04	5.22	4.95	4.36	6.44	-	0.90
alpha.-Farnesene	1505	1505	7.94	10.39	4.47	14.01	8.65	11.89	4.71	8.94	-	0.81
Alpha-Curcumene	1479	1480	-	-	-	-	-	-	-	-	-	3.14
Beta.-Sesquiphellandrene	1521	1522	16.07	14.91	18.00	18.66	18.10	8.48	20.24	21.78	-	2.70
Germacrene B	1559	1561	0.80	0.64	0.63	0.63	0.61	1.12	0.97	0.17	-	-
Beta.-Geraniol	1249	1242	0.35	0.98	0.08	1.52	0.79	-	-	1.13	-	
Beta.-Caryophyllene Epoxide	1582	1583	-	-	-	-	-	-	-	-	-	3.66
trans-Nerolidol	1561	1563	1.24	2.11	1.81	2.26	1.93	2.05	2.13	0.39	-	0.28
Elemol	1548	1549	0.64	0.51	-	0.47	-	-	-	-	-	-
beta.-Eudesmol	1649	1650	1.04	0.89	0.95	1.01	1.09	2.48	1.14	0.67	-	-
Farnesal	1740	1741	-	-	0.31	0.29	0.23	-	-	0.08	-	-
Unknown			12.75	10.50	11.44	11.74	14.20	24.19	15.24	11.30	15.68	52.46

Percentage of alpha limonene content varied from 0.41 % (Acc. 833) to 2.37 % (Gorubathane). Only in few genotypes *viz.,* black ginger (2.26 %), Mahim (1.16 %), red ginger (1.16 %) and Suruchi (1.04 %) this compounds content was >1 %.

Out of 30 genotypes, beta phellandrene was found to be 1.48 % in Maran and in other genotypes it was in very trace amount.

Furan was absent in ginger genotypes and present only in mango ginger which covered area percentage of 4.41.

Beta linalool is one of the major compounds present in black ginger with area percentage of 23.3. In case of other genotypes the content varied from 0.43 % (Karthika) to 1.71 % (Acc. 65).

In case of black ginger the number of chemical compounds detected was less as compared to other ginger genotypes. Number of compounds detected was only thirteen. Major compound in the essential oil of black ginger was beta linalool (23.30 %) followed by camphene (20.21 %), germacrene D (16.00 %) and Alpha pinene (13.17 %).

4.4 Development of DNA profiles of ginger genotypes

4.4.1 Assessing genetic diversity through RAPD and SSR markers

4.4.1.1 Molecular variability of ginger genotypes through RAPD

In RAPD analysis, polymorphic fragments were generated in ginger genotypes. The selection of primers was based on clear, scorable and reproducible amplified banding patterns.

Out of 30 primers used, 11 RAPD primers gave amplification and the number of amplification products obtained was specific to each primer. The size of the amplified products varied from 400 to 2800 bp. Of the 11 primers, ten primers *viz*., OPA 09, OPA 17, OPA 18, OPB 08, OPD 03, OPD 07, OPD 18, OPH 08, OPI 07 and OPL 12 were found to show 100 per cent polymorphism which is presented in Table 15. Of the 88 total alleles observed, 86 alleles were polymorphic and maximum numbers of 14 alleles were

Table 15: Polymorphism among ginger genotypes detected by RAPD markers

Primers	Total loci	MB	PB	% MM	% PM	Total amplicons	Amplicon range	PIC	Gene diversity
OPA 09	10	0	10	0	100	55	750-2600	0.981	0.816
OPA 17	6	0	6	0	100	80	1000-2500	0.985	0.555
OPA 18	8	0	8	0	100	98	400-1800	0.988	0.591
OPB 08	6	0	6	0	100	107	500-1500	0.996	0.448
OPD 03	3	0	3	0	100	87	1000-2000	0.998	0.275
OPD 07	7	0	7	0	100	92	1500-2300	0.998	0.561
OPD 18	9	0	9	0	100	169	500-2800	0.993	0.324
OPH 08	8	0	8	0	100	70	1200-2700	0.998	0.708
OPH 15	6	2	4	33.33	66.67	111	1000-2300	0.997	0.390
OPI 07	10	0	10	0	100	175	400-2600	0.993	0.358
OPL 12	14	0	14	0	100	253	400-2800	0.988	0.437
Total	**88**	**2**	**86**	**33.33**	**1066.67**	**1297**		**10.91**	**5.463**
Mean	**8**	**0.18**	**7.82**	**3.03**	**96.97**	**117.90**		**0.99**	**0.50**

MB – Number of Monomorphic Bands; PB – Number of Polymorphic Bands; % MM – Per cent Monomorphism; % PM – Per cent Polymorphism; PIC - Polymorphism Information Content

Table 16: Similarity matrix of ginger genotypes generated by RAPD analysis

	1	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
2	0.89	1.00																											
3	0.84	0.88	1.00																										
4	0.91	0.90	0.84	1.00																									
5	0.73	0.72	0.77	0.71	1.00																								
6	0.73	0.72	0.77	0.68	0.83	1.00																							
7	0.79	0.78	0.77	0.85	0.78	0.74	1.00																						
8	0.46	0.48	0.47	0.47	0.54	0.55	0.53	1.00																					
9	0.80	0.75	0.78	0.75	0.79	0.81	0.76	0.49	1.00																				
10	0.77	0.73	0.75	0.77	0.82	0.75	0.79	0.51	0.83	1.00																			
11	0.80	0.76	0.78	0.77	0.82	0.75	0.76	0.51	0.86	0.93	1.00																		
12	0.69	0.68	0.67	0.69	0.69	0.63	0.67	0.46	0.74	0.80	0.87	1.00																	
13	0.69	0.71	0.70	0.70	0.79	0.72	0.76	0.56	0.75	0.80	0.80	0.74	1.00																
14	0.69	0.71	0.68	0.70	0.73	0.69	0.73	0.53	0.69	0.80	0.80	0.74	0.92	1.00															
15	0.73	0.75	0.72	0.74	0.78	0.74	0.78	0.52	0.70	0.79	0.79	0.73	0.87	0.94	1.00														
16	0.48	0.48	0.47	0.47	0.60	0.55	0.54	0.43	0.52	0.54	0.54	0.46	0.63	0.63	0.61	1.00													
17	0.56	0.55	0.54	0.54	0.66	0.63	0.61	0.53	0.60	0.62	0.62	0.58	0.71	0.71	0.70	0.78	1.00												
18	0.56	0.57	0.59	0.56	0.69	0.69	0.64	0.44	0.63	0.65	0.65	0.61	0.71	0.71	0.73	0.74	0.71	1.00											
19	0.61	0.65	0.62	0.64	0.63	0.69	0.67	0.41	0.63	0.60	0.60	0.56	0.68	0.68	0.73	0.58	0.61	0.78	1.00										
20	0.56	0.55	0.54	0.52	0.60	0.60	0.59	0.38	0.60	0.56	0.56	0.52	0.65	0.65	0.67	0.69	0.60	0.80	0.74	1.00									
21	0.56	0.60	0.62	0.57	0.70	0.70	0.65	0.45	0.63	0.65	0.65	0.62	0.72	0.72	0.74	0.70	0.72	0.95	0.75	0.76	1.00								
22	0.59	0.58	0.57	0.57	0.67	0.61	0.62	0.42	0.63	0.63	0.63	0.58	0.72	0.69	0.71	0.66	0.64	0.80	0.75	0.90	0.77	1.00							
23	0.56	0.52	0.54	0.52	0.63	0.60	0.59	0.41	0.60	0.61	0.62	0.55	0.68	0.68	0.67	0.69	0.64	0.80	0.74	0.89	0.76	0.90	1.00						
24	0.50	0.49	0.51	0.48	0.63	0.57	0.53	0.45	0.56	0.58	0.58	0.51	0.61	0.58	0.57	0.74	0.72	0.72	0.57	0.68	0.73	0.73	0.72	1.00					
25	0.55	0.57	0.58	0.56	0.62	0.59	0.61	0.42	0.64	0.61	0.64	0.57	0.61	0.61	0.63	0.60	0.59	0.73	0.66	0.70	0.70	0.70	0.70	0.62	1.00				
26	0.59	0.58	0.57	0.55	0.63	0.64	0.62	0.42	0.63	0.60	0.60	0.56	0.65	0.62	0.64	0.58	0.57	0.72	0.68	0.85	0.69	0.85	0.80	0.64	0.70	1.00			
27	0.56	0.55	0.54	0.52	0.60	0.60	0.59	0.41	0.60	0.59	0.59	0.55	0.65	0.65	0.63	0.61	0.60	0.71	0.67	0.85	0.68	0.85	0.85	0.68	0.70	0.95	1.00		
28	0.34	0.36	0.37	0.35	0.42	0.38	0.35	0.39	0.38	0.40	0.40	0.37	0.38	0.36	0.35	0.35	0.36	0.42	0.37	0.36	0.40	0.40	0.39	0.40	0.59	0.38	0.39	1.00	
29	0.57	0.54	0.56	0.53	0.65	0.62	0.60	0.40	0.61	0.61	0.61	0.54	0.67	0.67	0.69	0.68	0.62	0.83	0.77	0.92	0.79	0.92	0.97	0.70	0.72	0.83	0.83	0.38	1.00
30	0.39	0.34	0.39	0.40	0.42	0.41	0.54	0.39	0.41	0.40	0.40	0.38	0.39	0.36	0.36	0.33	0.34	0.40	0.38	0.34	0.38	0.38	0.37	0.38	0.55	0.35	0.37	0.90	0.63

Note: 1-Suravi; 2-KAU Chandra; 3-Suruchi; 4-IISR Rejatha; 5-Aswathy; 6-RG 3; 7-Nadia; 8-Arunachal Pradesh local; 9-Acc. 65; 10-Suprabha; 11-Maran; 12-Rio de Janerio; 13-IISR Varada; 14-Acc. 833; 15-Mahim; 16-Acc.578; 17-Red ginger; 18-Acc. 219; 19-IISR Mahima; 20-Gorubathane; 21-Acc. 247; 22-Sourabh; 23-Himachal; 24-Karthika; 25-Bhaise;26-Mohini; 27-Athira; 28-Black ginger; 29-Zaheerabad local; 30-Mango ginger

obtained with primer OPL 12, followed by primer OPA 09 and OPI 07 with 10 alleles. Minimum numbers of 3 alleles were generated with primer OPD 03. Thus, amplifications varied across the primer employed. Among the 11 RAPD primers, the Polymorphism Information Content (PIC) was high in OPD 03, OPD 07 and OPH 08 (0.998)

Each RAPD pattern was compared with other patterns and genetic similarity matrix for all the thirty genotypes was constructed from binary data of markers using Jaccard's algorithm.

The coefficient of genetic similarity ranged from 39 - 97 per cent. Maximum similarity of 95 per cent was noticed between Himachal and Zaheerabad local (Table 16). Further, the information generated out of RAPD banding pattern was used for clustering through unweighted mean pair group arithmetic mean method (UPGMA).

Thirty ginger genotypes were used to study their genetic diversity through RAPD analysis using random primers**.** RAPD analysis of ginger genotypes with eleven random primersamplified DNA fragments with different sizes (Plate 8). The RAPD pattern obtained for these genotypes with different primers were defined by the presence or absence of bands. Each RAPD pattern was compared with each other and euclidean distance matrix was calculated for all the 30 ginger genotypes*.* The relationship among the genotypes was represented as dendrogram using UPGMA (Fig. 3). The genotypes were divided into two main groups, I and II sharing 39 % similarity which were further subdivided into clusters. Among the genotypes, two genotypes (black ginger and mango ginger) were grouped under group I with sharing similarity of 90 % and other 28 genotypes (Suravi, IISR Rejatha, KAU Chandra, Suruchi, Nadia, Aswathy, RG 3, Acc. 65, Suprabha, Maran, Rio de Janeiro, IISR Varada, Acc. 833, Mahim, Acc. 578, Red ginger, Karthika, Acc. 219, IISR Mahima, Gorubathane, Sourabh, Acc. 247, Mohini, Athira, Bhaise, Arunachal Pradesh local, Himachal and Zaheerabad local) were grouped under group II with sharing similarity of 47 %.

Group II consisted of two sub clusters namely A and B sharing similarity of 47 %. Cluster A consisted of one genotype *viz.,* Arunachal Pradesh local. Cluster B was sub

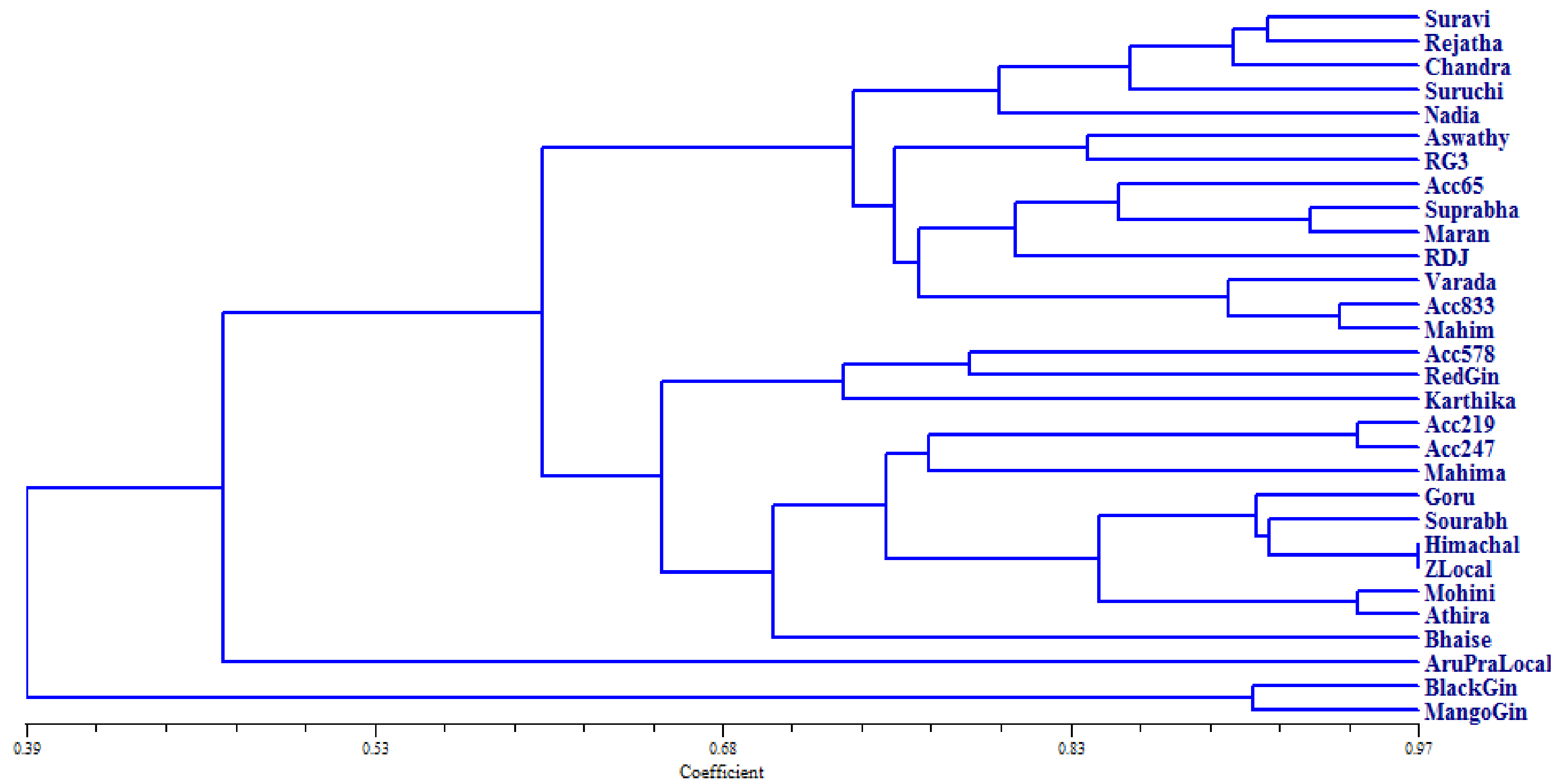
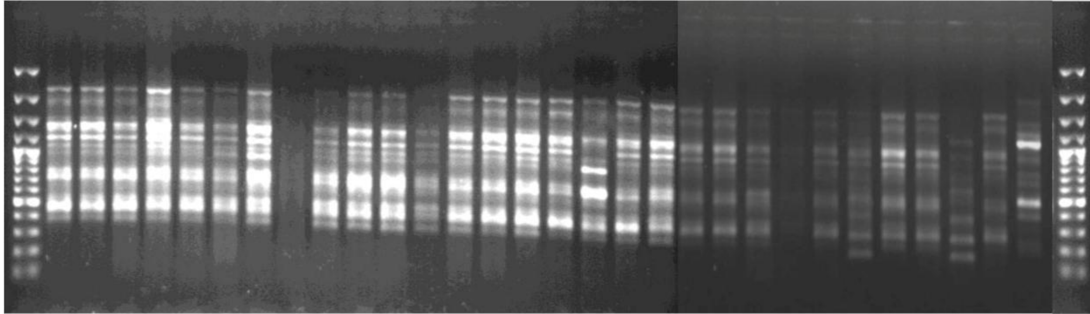


Fig. 3: UPGMA dendrogram based on RAPD markers using Jaccard's similarity coefficient

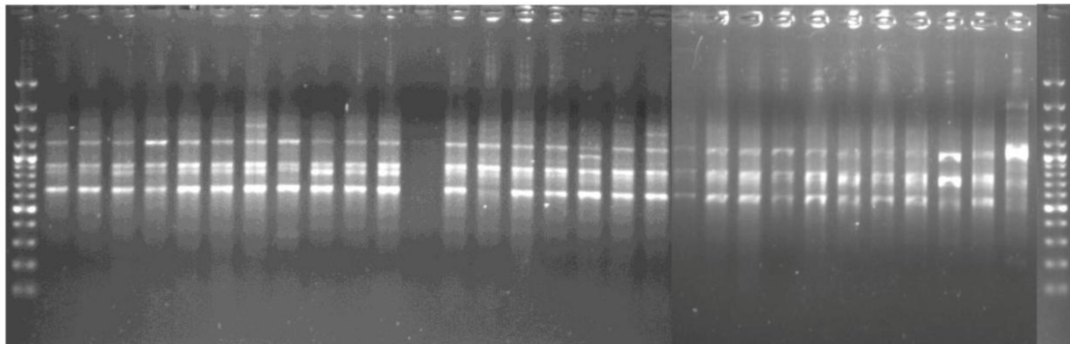
OPD 18

M 1 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 M



OPD 07

M 1 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 M



OPH 15

M 1 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 M

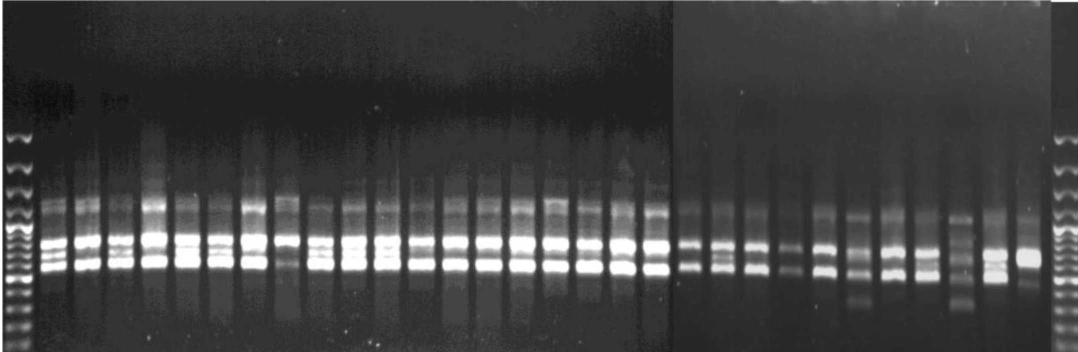


Plate 8: Amplification patterns of ginger genotypes with the selected RAPD primers

M: 1 Kb ladder; 1-Suravi; 2-KAU Chandra; 3-Suruchi;4-Rejatha; 5-Aswathy; 6- RG 3; 7-Nadia; 8-Arunachal local; 9-Acc. 65; 10-Suprabha; 11-Maran; 12-Rio de Janeiro; 13-Varada; 14-Acc. 833; 5-Mahim; 16-Acc. 578; 17-Red ginger; 18-Acc. 219; 19-Mahima; 20-Gorubathane; 21-Acc. 247; 22-Sourabh; 23-Himachal; 24-Karthika; 25-Bhaise; 26-Mohini; 27-Athira; 28-Black ginger; 9-Zaheerabad local; 30-Mango ginger

divided into C and D sharing similarity of 60 %. Group C further divided into cluster E and F sharing approximately 65 % similarity. Cluster E was subdivided into G & H sharing 70 % similarity. Cluster G consisted only one genotype Bhaise. Cluster H consisted of nine genotypes (Acc. 219, Acc. 247, Mahima, Gorubathane, Sourabh, Himachal, Zaheerabad local, Mohini and Athira). Among the nine genotypes, Himachal and Zaheerabad local showed 97 % similarity followed by 94 % similarity was observed between Mohini and Athira as well as Acc. 219 and Acc. 247. Cluster F consisted of three genotypes *viz.,* Acc. 578, red ginger and Karthika showing 71 % similarity.

Cluster D is subdivided into 2 clusters I and J. Cluster I consisted of nine genotypes such as Aswathy, RG 3, Acc. 65, Suprabha, Maran, Rio de Janeiro, Varada, Acc. 833 and Mahim. In cluster I maximum similarity of approximately 93 % was noticed between the genotypes Acc. 833 and Mahim. Cluster J consisted of five genotypes (Suravi, IISR Rejatha, KAU Chandra, Suruchi and Nadia) sharing approximately 78 % similarity.

4.4.1.2 Molecular variability of ginger genotypes through SSR

Out of 55 SSR primers screened, sixteen primers amplified and produced 34 alleles among them 25 were polymorphic bands and 10 were monomorphic bands. SSR fragments ranged from 100 to 1200 bp in size (Table 17).

Maximum number of alleles detected was seven from ZOM 103 primer. With the average of 62.80 per cent polymorphism produced by sixteen SSR primers, cent per cent polymorphism was detected by the primers ZOC 11, ZOC 28, ZOC 156, ZOC 33, ZOM 064, ZOM 140 and CLEST 16. Polymorphism information content (PIC), a measure of gene diversity was an average of 0.92 with a range of 0.889 by ZOM 033 to 0.982 by CLEST 16 primer.

Jaccard's similarity coefficients among the thirty genotypes helped to establish genetic relationships (Fig. 4). Phylogenetic analyses of thirty genotypes, conducted on SSR banding patterns, indicated that maximum percentage of similarity (100 %) was observed between KAU Chandra, IISR Mahima & Mohini; IISR Rejatha and Nadia; Acc

Table 17: Polymorphism among ginger genotypes detected by SSR markers

Primers	No. of alleles	MB	PB	% MM	% PM	Total amplicons	Allele range (bp)	PIC	Gene diversity
ZOC 11	2	0	2	0	100	30	240-250	0.893	0.333
ZOC 28	3	0	3	0	100	31	150-280	0.923	0.655
ZOC 92	1	1	0	100	0	30	190	0.943	0
ZOC 98	3	1	2	33.33	66.66	88	250-280	0.952	0.022
ZOC 100	2	1	1	50	50	58	150-170	0.922	0.033
ZOC 156	3	0	3	0	100	36	150-250	0.897	0.60
ZOC 33	1	0	1	0	100	29	180	0.889	0.633
ZOM 040	2	1	1	50	50	42	190-210	0.921	0.3
ZOM 055	1	1	0	100	0	30	190	0.921	0
ZOM 064	1	0	1	0	100	28	250	0.954	0.066
ZOM 103	7	2	5	28.57	71.43	101	150-1200	0.988	0.545
ZOM 107	3	1	2	33.33	66.66	32	190-400	0.893	0.644
ZOM 111	1	1	0	100	0	30	300	0.906	0
ZOM 140	2	0	2	0	100	58	140-150	0.940	0.033
CLEST 15	1	1	0	100	0	30	150	0.948	0
CLEST 16	2	0	2	0	100	56	170-190	0.982	0.066
Total	**35**	**10**	**25**	**595.23**	**1004.75**	**709**		**14.87**	**3.597**
Mean	**2.18**	**0.62**	**1.56**	**37.20**	**62.80**	**44.31**		**0.92**	**0.22**

MB – Number of Monomorphic Bands; PB – Number of Polymorphic Bands; % MM – Per cent Monomorphism; % PM – Per cent Polymorphism; PIC - Polymorphism Information Content

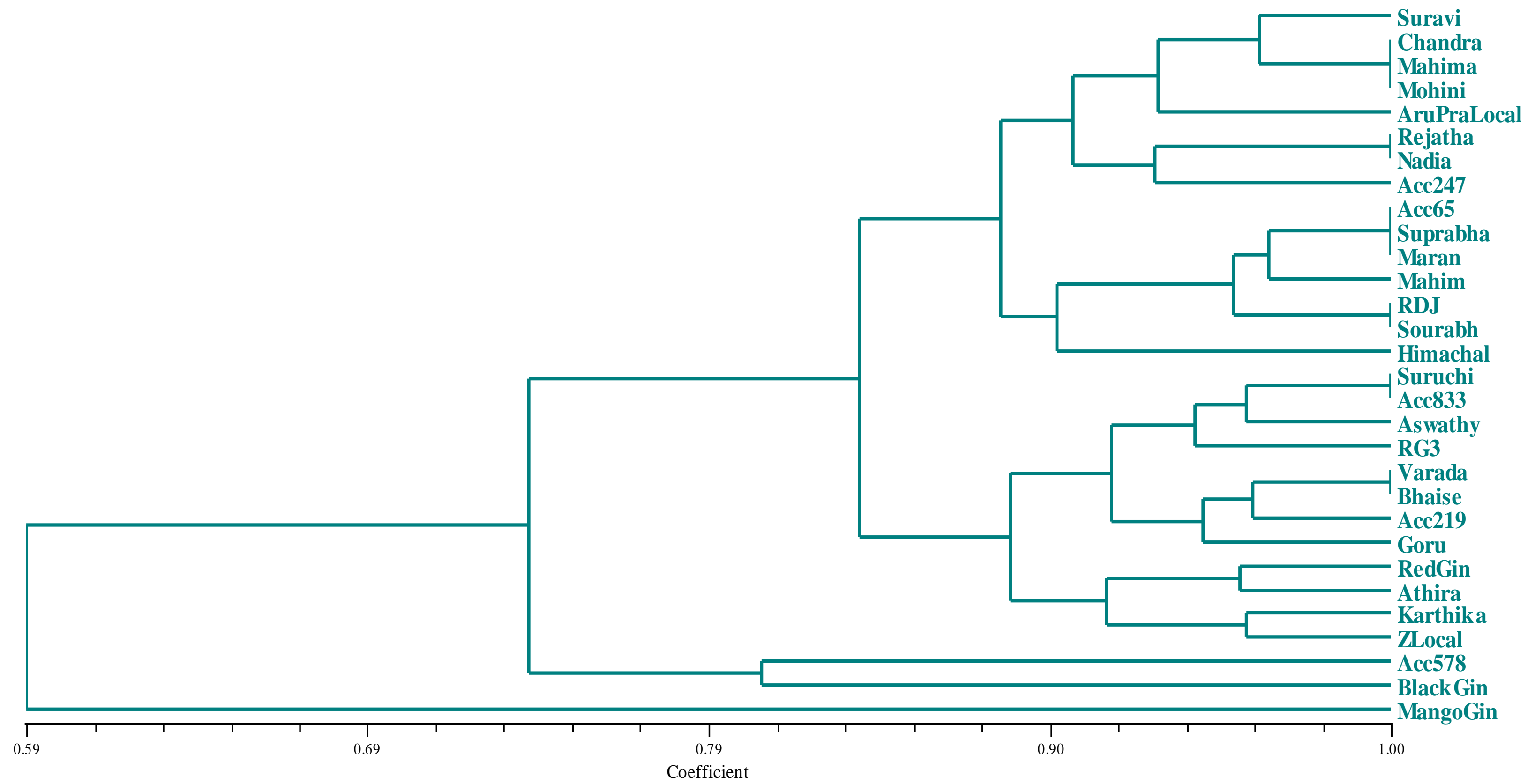


Fig. 4: UPGMA dendrogram based on SSR markers using Jaccard's similarity coefficient

65, Suprabha and Maran; Rio de Janeiro and Sourabh; Suruchi and Acc. 833; IISR Varada and Bhaise (Table 18).

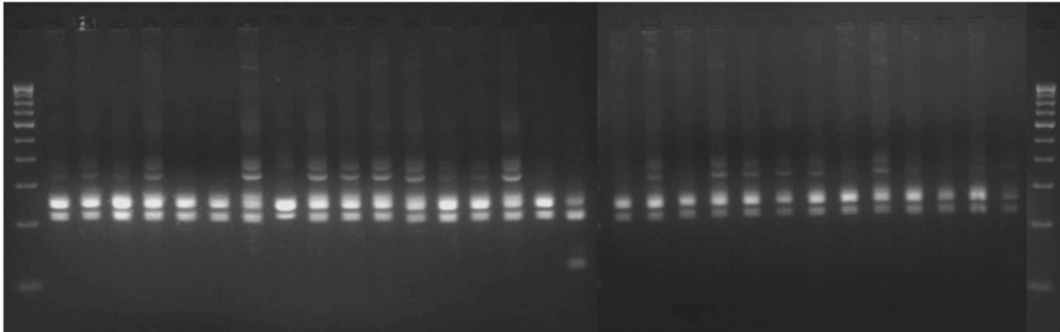
Thirty ginger genotypes were used to study their variability through SSR analysis using sixteen primers(Plate 9). The SSR pattern obtained for these genotypes with different primers were defined by the presence or absence of bands. Each SSR pattern was compared with each other and euclidean distance matrix was calculated for all the 30 ginger genotypes. The relationship among the genotypes was represented as dendrogram using UPGMA.

The genotypes were divided into two main groups, I and II sharing 59 % similarity. Group I comprised of only one genotype, mango ginger. Group II was further subdivided into cluster A and B with similarity percentage of 74. Cluster A consisted of 2 genotypes (Acc. 578 and black ginger) sharing similarity of approximately 81 %. Cluster B was subdivided into 2 clusters C and D sharing percentage similarity of 84 %. Cluster C divided into 2 sub clusters E and F with 89 % similarity. Cluster E consisted of 4 genotypes namely Red ginger, Athira, Karthika and Zaheerabad local sharing 92 % similarity. Cluster F consisted of 8 genotypes *viz.,* Suruchi, Acc. 833, Aswathy, RG 3, IISR Varada, Bhaise, Acc. 219 and Gorubathane. Among the 8 genotypes Suruchi and Acc. 833 shared 100 % similarity; IISR Varada and Bhaise were also 100 % similar to each other.

Cluster D was subdivided into 2 clusters namely G and H with similarity percentage of approximately 88 %. Cluster G consisted of 7 genotypes sharing 91 % similarity, among 7 genotypes Acc. 65, Suprabha and Maran showed 100 % similarity and Rio de Janeiro and Sourabh were also 100 % similar. Cluster H consisted 8 genotypes sharing 91 % similarity, among them genotypes KAU Chandra, IISR Mahima and Mohini were 100 % similar. Similarly, genotypes IISR Rejatha and Nadia also showed 100 % similarity.

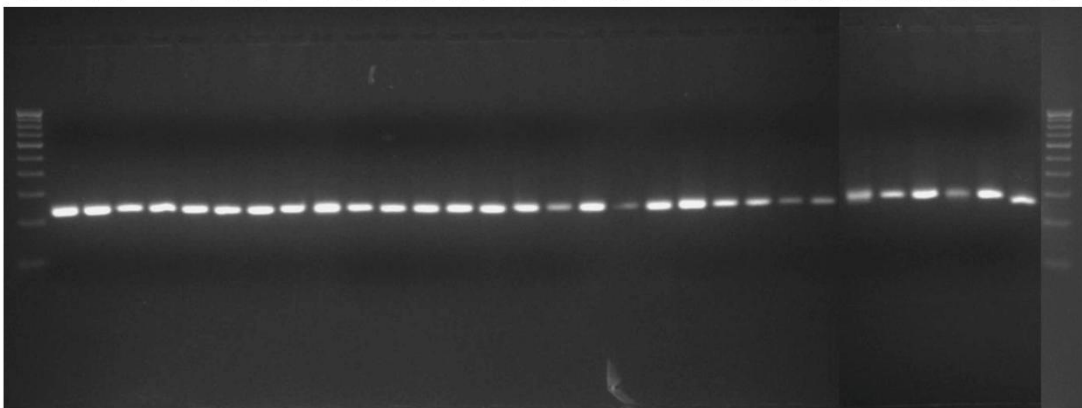
ZOM 103

M 1 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 M



ZOC 11

M 1 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 M



ZOC 28

M 1 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 M

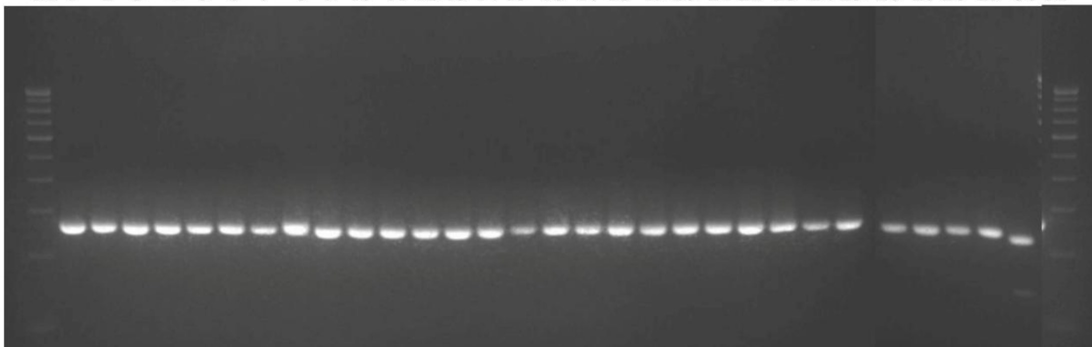


Plate 9: Amplification patterns of ginger genotypes with the selected SSR primers

M: 1 Kb ladder; 1-Suravi; 2-KAU Chandra; 3-Suruchi;4-Rejatha; 5-Aswathy; 6- RG 3; 7-Nadia; 8-Arunachal local; 9-Acc. 65; 10-Suprabha; 11-Maran; 12-Rio de Janeiro; 13-Varada; 14-Acc. 833; 15-Mahim; 16-Acc. 578; 17-Red ginger; 18-Acc. 219; 19-Mahima; 20-Gorubathane; 21-Acc. 247; 22-Sourabh; 23-Himachal; 24-Karthika; 25-Bhaise; 26-Mohini; 27-Athira; 28-Black ginger; 29-Zaheerabad local; 30-Mango

Table 18: Similarity matrix of ginger genotypes generated by SSR analysis

	1	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
2	0.96	1.00																											
3	0.92	0.88	1.00																										
4	0.89	0.93	0.81	1.00																									
5	0.88	0.85	0.96	0.79	1.00																								
6	0.88	0.84	0.95	0.78	0.91	1.00																							
7	0.89	0.93	0.81	1.00	0.79	0.78	1.00																						
8	0.96	0.92	0.88	0.85	0.84	0.83	0.85	1.00																					
9	0.92	0.89	0.85	0.83	0.84	0.81	0.83	0.88	1.00																				
10	0.92	0.89	0.85	0.83	0.88	0.81	0.83	0.88	1.00	1.00																			
11	0.92	0.89	0.85	0.83	0.88	0.81	0.83	0.88	1.00	1.00	1.00																		
12	0.96	0.92	0.88	0.86	0.85	0.84	0.86	0.92	0.96	0.96	0.96	1.00																	
13	0.88	0.92	0.96	0.85	0.92	0.91	0.85	0.84	0.81	0.81	0.81	0.85	1.00																
14	0.92	0.88	1.00	0.81	0.96	0.95	0.81	0.88	0.85	0.85	0.85	0.88	0.96	1.00															
15	0.89	0.86	0.81	0.86	0.85	0.78	0.86	0.85	0.96	0.96	0.96	0.26	0.79	0.81	1.00														
16	0.76	0.80	0.83	0.74	0.79	0.78	0.74	0.72	0.70	0.70	0.70	0.73	0.87	0.83	0.68	1.00													
17	0.84	0.81	0.91	0.75	0.79	0.87	0.75	0.80	0.78	0.78	0.78	0.81	0.88	0.91	0.75	0.83	1.00												
18	0.85	0.88	0.92	0.82	0.88	0.88	0.82	0.81	0.85	0.85	0.85	0.88	0.96	0.92	0.82	0.83	0.84	1.00											
19	0.96	1.00	0.88	0.93	0.85	0.84	0.93	0.92	0.89	0.89	0.89	0.92	0.92	0.88	0.86	0.80	0.81	0.88	1.00										
20	0.84	0.88	0.91	0.81	0.88	0.87	0.81	0.80	0.78	0.78	0.78	0.81	0.96	0.91	0.75	0.91	0.91	0.92	0.88	1.00									
21	0.89	0.93	0.81	0.93	0.79	0.78	0.93	0.85	0.89	0.89	0.89	0.93	0.85	0.81	0.93	0.74	0.75	0.89	0.93	0.81	1.00								
22	0.96	0.92	0.88	0.86	0.85	0.84	0.86	0.92	0.96	0.96	0.96	1.00	0.85	0.88	0.93	0.73	0.81	0.88	0.92	0.81	0.93	1.00							
23	0.89	0.86	0.81	0.80	0.79	0.78	0.80	0.85	0.89	0.89	0.89	0.93	0.79	0.81	0.86	0.68	0.75	0.82	0.86	0.75	0.86	0.93	1.00						
24	0.96	0.92	0.88	0.85	0.84	0.83	0.85	0.92	0.88	0.88	0.88	0.92	0.84	0.88	0.85	0.79	0.88	0.81	0.92	0.88	0.85	0.92	0.85	1.00					
25	0.88	0.92	0.96	0.85	0.92	0.91	0.85	0.84	0.81	0.81	0.81	0.85	1.00	0.95	0.79	0.87	0.88	0.96	0.92	0.96	0.85	0.85	0.79	0.84	1.00				
26	0.96	1.00	0.88	0.93	0.85	0.84	0.93	0.92	0.89	0.89	0.89	0.92	0.92	0.88	0.86	0.80	0.81	0.88	1.00	0.88	0.93	0.92	0.86	0.92	0.92	1.00			
27	0.88	0.84	0.95	0.78	0.91	0.91	0.78	0.83	0.81	0.81	0.81	0.84	0.91	0.95	0.78	0.86	0.95	0.88	0.84	0.95	0.78	0.84	0.78	0.91	0.91	0.84	1.00		
28	0.68	0.72	0.74	0.67	0.71	0.70	0.67	0.64	0.63	0.63	0.63	0.65	0.78	0.74	0.61	0.81	0.74	0.75	0.72	0.82	0.67	0.65	0.61	0.71	0.78	0.72	0.77	1.00	
29	0.92	0.88	0.91	0.81	0.88	0.87	0.81	0.88	0.85	0.85	0.85	0.80	0.88	0.91	0.81	0.83	0.91	0.84	0.88	0.91	0.81	0.88	0.81	0.96	0.88	0.88	0.95	0.74	1.00
30	0.62	0.59	0.60	0.55	0.58	0.56	0.55	0.58	0.57	0.57	0.57	0.59	0.58	0.60	0.55	0.58	0.60	0.56	0.59	0.60	0.51	0.59	0.55	0.64	0.58	0.59	0.63	0.64	0.67

Note: 1-Suravi; 2-KAU Chandra; 3-Suruchi; 4-IISR Rejatha; 5-Aswathy; 6-RG 3; 7-Nadia; 8-Arunachal Pradesh local; 9-Acc. 65; 10-Suprabha; 11-Maran; 12-Rio de Janerio; 13-IISR Varada; 14-Acc. 833; 15-Mahim; 16-Acc.578; 17-Red ginger; 18-Acc. 219; 19-IISR Mahima; 20-Gorubathane; 21-Acc. 247; 22-Sourabh; 23-Himachal; 24-Karthika; 25-Bhaise; 26-Mohini; 27-Athira; 28-Black ginger; 29-Zaheerabad local; 30-Mango ginger

4.4.1.3 Molecular variability of ginger genotypes through pooled RAPD and SSR markers

The data obtained on RAPD and SSR primers were pooled to assess the polymorphism (Table 19).

Data obtained from pooled analysis of RAPD and SSR primers revealed that, the ginger genotypes were divided into 2 main groups I and II sharing 49 % similarity (Fig. 5). Group I consisted of only one genotype black ginger. Group II was further subdivided into 2 clusters A and B sharing approximately 50 % similarity. Cluster A consisted of only one genotype *i.e.* Mango ginger. Cluster B further divided into cluster B and D with 53 % similarity. Cluster C consisted of two genotypes (Himachal and Zaheerabad local) sharing approximately 63 % similarity. Cluster D is subdivided into cluster E and F sharing similarity percentage of 68. Cluster E was subdivided into G and H with 72 % similarity. Cluster G consisted of eight genotypes namely Acc. 219, IISR Mahima, Gorubathane, Mohini, Athira, Acc 247, Sourabh and Bhaise. Among them, Acc. 247 and Sourabh showed maximum similarity of 90 %. Cluster H consisted of three genotypes, Acc. 578, Red ginger and Karthika sharing 77 % similarity. Cluster F was divided into 2 clusters, I and J sharing 77 % similarity. Cluster I consisted of nine genotypes namely Aswathy, RG 3, Acc. 65, Suprabha, Maran, Rio de Janeiro, IISR Varada, Acc. 833 and Mahim. Among them, genotypes Suprabha and Maran showed 100 % similarity. Cluster J consisted of 5 genotypes (Suravi, KAU Chandra, IISR Rejatha, Suruchi and Nadia) sharing approximately 83 % similarity.

4.4.1.4 Comparison of RAPD and SSR marker systems for their efficacy in assessing genetic diversity of ginger genotypes

To compare the utility of the two marker systems, thirty ginger genotypes were analyzed with eleven RAPD and sixteen SSR primers. Various parameters *viz*., total number of alleles, number of polymorphic bands per assay unit, mean percentage of polymorphism per assay, number of monomorphic bands per assay and polymorphic information content (PIC) value were recorded as criteria to differentiate their efficacy and the results are presented in Table 20.The mean number of allele per assay unit,

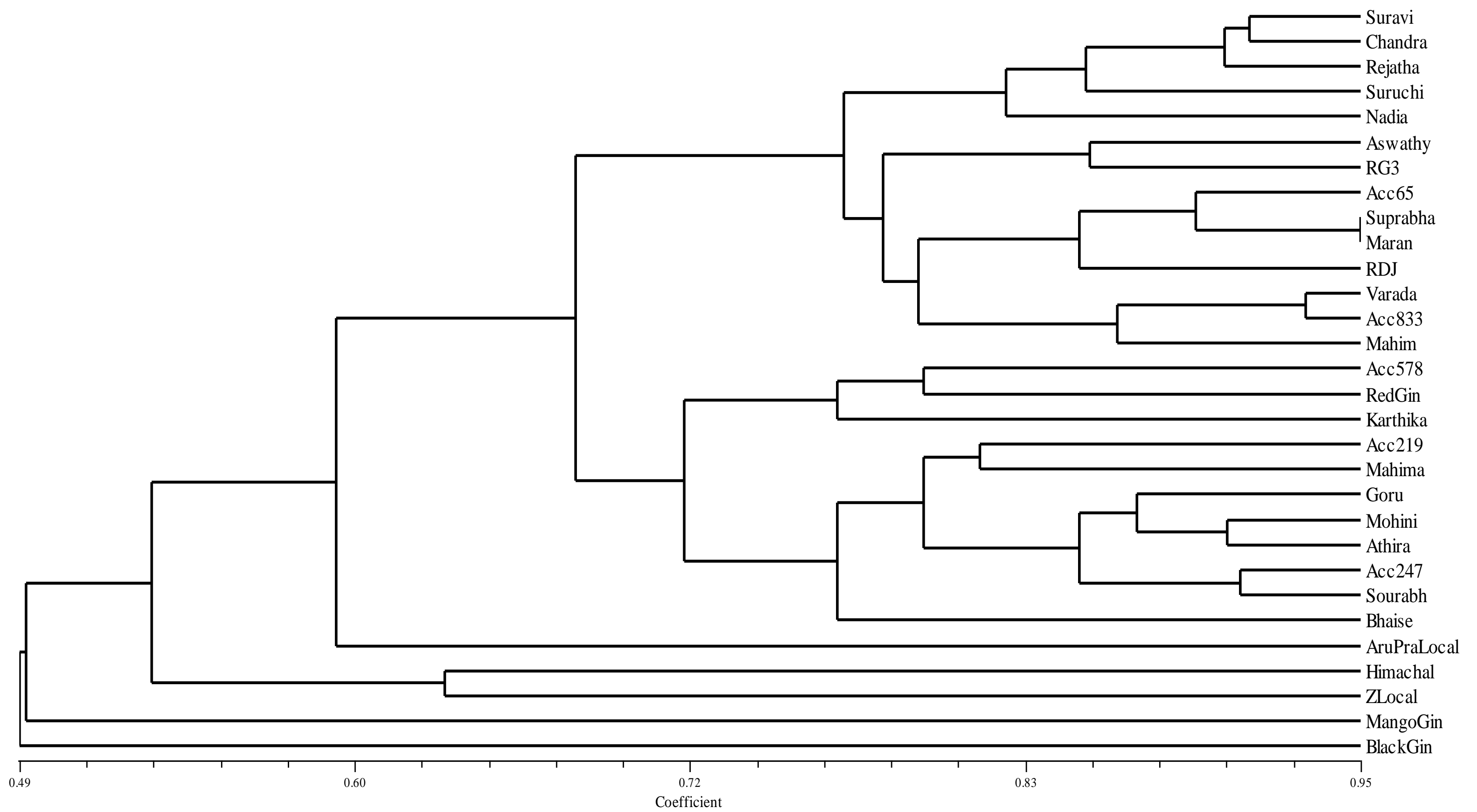


Fig. 5: UPGMA dendrogram based on RAPD and SSR markers using Jaccard's similarity coefficient

Table 19: Similarity matrix of ginger genotypes generated by RAPD and SSR analysis

	1	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
2	0.91	1.00																											
3	0.86	0.88	1.00																										
4	0.90	0.90	0.83	1.00																									
5	0.77	0.76	0.82	0.73	1.00																								
6	0.77	0.75	0.81	0.71	0.86	1.00																							
7	0.82	0.82	0.78	0.89	0.78	0.75	1.00																						
8	0.60	0.60	0.58	0.58	0.64	0.63	0.64	1.00																					
9	0.84	0.78	0.80	0.77	0.82	0.81	0.78	0.62	1.00																				
10	0.81	0.78	0.78	0.79	0.84	0.77	0.80	0.63	0.88	1.00																			
11	0.83	0.80	0.80	0.79	0.84	0.77	0.78	0.63	0.90	0.95	1.00																		
12	0.76	0.75	0.73	0.74	0.74	0.69	0.73	0.61	0.81	0.85	0.90	1.00																	
13	0.74	0.77	0.77	0.74	0.83	0.78	0.79	0.65	0.68	0.81	0.81	0.78	1.00																
14	0.75	0.76	0.76	0.73	0.79	0.77	0.76	0.64	0.74	0.82	0.82	0.78	0.93	1.00															
15	0.78	0.78	0.75	0.78	0.80	0.75	0.81	0.63	0.78	0.85	0.85	0.79	0.84	0.90	1.00														
16	0.56	0.57	0.56	0.55	0.67	0.62	0.60	0.54	0.58	0.59	0.59	0.55	0.70	0.69	0.64	1.00													
17	0.64	0.62	0.64	0.60	0.73	0.70	0.66	0.63	0.65	0.67	0.67	0.65	0.77	0.78	0.72	0.80	1.00												
18	0.64	0.66	0.68	0.64	0.76	0.75	0.70	0.57	0.70	0.72	0.72	0.70	0.79	0.78	0.77	0.77	0.76	1.00											
19	0.71	0.75	0.69	0.72	0.70	0.73	0.75	0.57	0.71	0.69	0.69	0.67	0.75	0.74	0.77	0.66	0.68	0.82	1.00										
20	0.64	0.64	0.64	0.60	0.68	0.68	0.66	0.52	0.65	0.63	0.63	0.61	0.74	0.73	0.70	0.77	0.71	0.84	0.79	1.00									
21	0.66	0.64	0.62	0.64	0.68	0.66	0.70	0.57	0.69	0.71	0.71	0.68	0.74	0.73	0.76	0.71	0.68	0.84	0.81	0.86	1.00								
22	0.69	0.68	0.66	0.66	0.73	0.68	0.70	0.59	0.73	0.73	0.73	0.72	0.76	0.75	0.78	0.69	0.70	0.84	0.81	0.86	0.91	1.00							
23	0.47	0.46	0.45	0.44	0.53	0.49	0.48	0.50	0.51	0.54	0.52	0.50	0.52	0.53	0.54	0.57	0.55	0.63	0.56	0.60	0.65	0.65	1.00						
24	0.63	0.61	0.61	0.59	0.70	0.65	0.63	0.62	0.67	0.68	0.68	0.64	0.69	0.68	0.67	0.76	0.78	0.75	0.69	0.75	0.77	0.80	0.61	1.00					
25	0.64	0.66	0.68	0.64	0.71	0.68	0.68	0.55	0.70	0.67	0.69	0.66	0.72	0.71	0.68	0.69	0.68	0.81	0.74	0.78	0.75	0.75	0.59	0.70	1.00				
26	0.69	0.70	0.66	0.66	0.71	0.70	0.72	0.59	0.71	0.69	0.69	0.68	0.74	0.71	0.72	0.66	0.66	0.78	0.79	0.86	0.85	0.88	0.60	0.75	0.77	1.00			
27	0.64	0.63	0.65	0.59	0.69	0.69	0.65	0.55	0.66	0.66	0.66	0.64	0.73	0.74	0.68	0.70	0.72	0.77	0.73	0.89	0.82	0.85	0.58	0.76	0.77	0.90	1.00		
28	0.43	0.46	0.47	0.44	0.51	0.47	0.44	0.48	0.46	0.47	0.47	0.62	0.50	0.47	0.44	0.50	0.49	0.54	0.48	0.51	0.49	0.49	0.53	0.52	0.66	0.49	0.51	1.00	
29	0.45	0.44	0.45	0.43	0.51	0.49	0.47	0.46	0.48	0.49	0.49	0.45	0.51	0.51	0.49	0.56	0.56	0.60	0.53	0.59	0.59	0.59	0.63	0.69	0.58	0.59	0.63	0.52	1.00
30	0.46	0.45	0.46	0.45	0.49	0.44	0.47	0.43	0.47	0.49	0.49	0.49	0.47	0.49	0.48	0.55	0.51	0.52	0.49	0.53	0.52	0.52	0.43	0.56	0.56	0.52	0.54	0.45	0.41

Note: 1-Suravi; 2-KAU Chandra; 3-Suruchi; 4-IISR Rejatha; 5-Aswathy; 6-RG 3; 7-Nadia; 8-Arunachal Pradesh local; 9-Acc. 65; 10-Suprabha; 11-Maran; 12-Rio de Janerio; 13-IISR Varada; 14-Acc. 833; 15-Mahim; 16-Acc.578; 17-Red ginger; 18-Acc. 219; 19-IISR Mahima; 20-Gorubathane; 21-Acc. 247; 22-Sourabh; 23-Himachal; 24-Karthika; 25-Bhaise; 26-Mohini; 27-Athira; 28-Black ginger; 29-Zaheerabad local; 30-Mango ginger

Table 20: Comparative analysis of banding patterns generated by RAPD and SSR

Components	RAPD	SSR
Number of alleles per assay unit	11	16
Total amplicons	1297	709
Total number of alleles	88	35
Mean number of allele per assay unit	8	2.18
Number of polymorphic band per assay unit	7.82	1.56
Mean (%) polymorphism per assay	96.97	62.80
Number of monomorphic band per assay unit	0.18	0.62
Mean PIC per assay	0.99	0.92

number of polymorphic and monomorphic bands per assay unit in SSR analysis was 16.0, 1.56 and 0.62 respectively, and in case of RAPD primers it was 11.0, 7.82 and 0.18 respectively. Mean percentage of polymorphism per assay was 96.97 % in RAPD, whereas, it is 62.80 % in case of SSR primers.

4.4.2 Identification of SNPs from RNA-seq Data

4.4.2.1 Filtering of raw reads

Five transcriptome data were subjected to NGS quality check by using the tool NGSQC Toolkit, SQIT. After the quality filtering, Queensland transcriptome with 4,76,93,985 reads and average read length of 150.0 bp; IISR Varada with 1,46,30,845 reads and average read length of 101.0 bp; Maran with 3,09,70,586 reads having average read length of 72.0 bp; Yujiang 1 with 5,57,20,210 reads and 149.25 bp average read length; Southwest with 2,56,20,582 reads and average read length of 150.0 bp were selected for comparative transcriptome analysis for SNPs detection (Table 21).

4.4.2.2 SNP calling

KisSplice was carried out with default parameters with five conditions, using IISR Varada as reference transcriptome to identify SNPs of other four samples. TransDecoder software was used to predict the ORFs and the amino acid sequence of the transcripts. BLAT was used to position the predicted SNPs onto the assembled transcripts. The number of SNPs identified is presented in table 22.

61,424 common transcripts were obtained when the transcriptome of Queensland and IISR Varada transcriptomes compared, out of which 33,694 SNPs were identified in coding sequences and 27,730 SNPs in non coding sequences. Among the SNPs in the coding sequences, 19,268 were synonymous and 14,426 were non synonymous. Similarly total of 43,635 similar transcripts were detected in the comparison of Maran and IISR Varada transcriptomes, out of which 27,863 SNPs were in coding sequence and 15,772 SNPs were in non-coding sequences. Out of 27,863 SNPs obtained, 16,657 SNPs were synonymous and 11,206 SNPs were non synonymous.

Table 21: Summary of *Zingiber officinale* transcripts base quality

Genotype	Trascriptome identity	Raw reads (Before NGSQC)	Raw reads (After NGSQC)	Percentage high quality reads	Average read length (bp)
Queensland	G1-HMCH	59800257	47693985	79.76	150.00
IISR Varada	SRR3268680	24107482	14630845	60.70	101.00
Maran	SRR1924263	38310159	30970586	80.84	72.00
Yujiang 1	SRR5313727	64406628	55720210	86.51	149.25
Southwest	SRR5512053	27645008	25620582	92.68	150.00

Table 22: Summary of detected SNPs

Genotype comparison	Number of transcripts	SNPs in CDS	SNPs not in CDS	Synonymous change	Non synonymous change
Queensland V/s IISR Varada	61424	33694	27730	19268	14426
Maran V/s IISR Varada	43635	27863	15772	16657	11206
Yujiang 1 V/s IISR Varada	59081	33011	26070	19261	13750
Southwest V/s IISR Varada	56194	31983	24211	18678	13305

Total number of similar transcripts obtained by comparison of Yujiang 1 and IISR Varada transcriptomes were 59,081 and out of which 33,011 SNPs in CDS and 26,070 SNPs in non coding sequences. Out of 33,011 SNPs in CDS, 19,261 SNPs were synonymous and 13,750 were non synonymous. In case of comparison of transcriptomes of Southwest and IISR Varada a total of 56,194 similar transcripts were detected, out of which 31,983 SNPs were in coding sequence and 24,211 SNPs were not in non coding regions. Out of 31,983 SNPs obtained in CDS, 18,678 SNPs were synonymous and 13,305 SNPs were non synonymous.

V. DISCUSSION

Ginger (*Zingiber officinale* Rosc.) is an important rhizomatous spice produced and exported from India. India is one of the largest producer and exporter of ginger. It is one of the oldest and most important spices, being cultivated in Tropical Asia for over 3000 years (Purseglove *et al*., 1981). It is one of the earliest oriental spices known to Europe and is still in large demand today (Parthasarathy *et al.,* 2012). Dried rhizomes are esteemed for their aroma, flavour and pungency (Purseglove *et al.,* 1981). Rhizome of ginger has been used as a medicine in Chinese, Indian and Arabic herbal traditions since ancient times (Atman and Marcussen, 2001).

Scope to improve any crop depends on the magnitude of genetic variability present in the germplasm collections. Greater the variability in the germplasm, better would be the chances of selecting superior genotypes (Simmonds, 1962). Several cultivars of ginger are grown in different ginger growing areas of India and they are generally named after the localities where they are grown. Breeding of ginger through selection and hybridization is seriously handicapped by lack of variability, absence of natural seed set and exclusive vegetative propagation. Sexual reproduction is not reported in ginger, however the geographical spread accompanied by genetic differentiation into locally adapted population augmented by mutation is the main factor responsible for diversity in this clonally propagated crop (Parthasarathy *et al.* 2011). Ginger genotypes are identified based on morphological traits. Characterization of the ginger genotypes based on certain morphological traits which are not altered by the environmental interactions will be of greater help for easy identification of the genotypes.

Molecular markers are proved to be valuable tools in the characterisation and evaluation of genetic diversity within and between species and populations. Molecular markers are the most powerful tools available for discerning biosystematic, biogeographic and phylogenetic relationships. Molecular markers, unlike morphological markers are stable and have been found to be useful in population studies, phylogeny, stability testing and genetic mapping.

The biochemical characterization is helpful to detect differences as well as identifying the varieties for different end use.

Hence, in the present study, a combination of morphological, biochemical and molecular techniques have been used to characterize the ginger genotypes. Parallely, SNP identification using comparative transcriptome approach is also carried out to identify the potential SNPs which can be further used in genotyping of ginger. The results obtained are discussed hereunder.

5.1 Studies on genetic diversity in ginger

5.1.1 Morphological and rhizome characters of ginger genotypes

Significant differences were observed among the genotypes for all the characters *viz.,* plant height, number of tillers, number of leaves on main shoot, total number of leaves, leaf width, leaf area, shoot diameter, rhizome thickness and yield parameters except for leaf length.

During 2016/17, highest plant height was recorded in Acc. 833 and the genotypes Maran and IISR Varada were on par with Acc. 833. More number of tillers per clump was recorded in Mahim. Higher shoot diameter and more number of leaves on main shoot was recorded by the genotype Maran. More number of leaves per clump was recorded by Mahim. Higher leaf width and leaf area were recorded by IISR Varada. Bhaise recorded higher rhizome thickness.

In the year 2017/18, highest plant height was recorded in Suravi which was on par with Maran, Gorubathane, Nadia, Bhaise, Himachal, IISR Varada, IISR Rejatha, Sourabh and KAU Chandra. In the 2nd year also Mahim recorded more number of tillers and Aswathy recorded higher shoot diameter. More number of leaves on main shoot was observed by IISR Varada. More number of leaves per clump was recorded in Suravi. Highest leaf width and leaf area are recorded by Bhaise and RG 3, respectively. In the subsequent year also Bhaise recorded higher rhizome thickness.

From the pooled mean analysis it was observed that the genotype Maran recorded the highest plant height, which was on par with Gorubathane, IISR Varada, Suravi and Nadia. The genotype Mahim recorded maximum number of tillers per clump which was on par with Suravi and Suprabha. Shoot diameter was highest in Aswathy and was on par with IISR Varada, Suravi, Athira, Sourabh, Maran, Gorubathane, Acc. 219, RG 3 and red ginger. Number of leaves on main shoot was maximum in IISR Varada and the genotypes Maran, Gorubathane, Nadia, Red ginger, Sourabh, Acc. 219, Acc. 833, Zaheerabad local, IISR Rejatha and Acc. 247 were on par with IISR Varada. Leaf width and leaf area was highest in RG 3 and the genotypes IISR Mahima, Suprabha, Suruchi, Aswathy, Maran, Himachal, Bhaise, Zaheerabad local, Acc. 247, Acc. 65, Acc. 219, Acc. 833 and Red ginger were on par. Rhizome thickness was higher in Bhaise and genotypes Aswathy, Zaheerabad local and Acc. 247 were on par with Bhaise. In general considerable variability was recorded for different characters and could be due to genetic constituent of the genotypes with reaction to environment *i.e.* genotype x environment interaction. Such variations in plant growth parameters have been reported by earlier workers Jyotsna *et al.* (2012), Shetty *et al.* (2015), Babu *et al.* (2017), Balakumbahan and Joshua (2017) and Goudar *et al.* (2017) in ginger.

During both the years also genotype Maran recorded highest yield per plant and projected yield and the genotypes Acc. 247, Himachal, IISR Varada, Karthika, Aswathy were on par with Maran.

Pooled mean analysis exhibited that the genotypes Maran, Acc. 247, Himachal and IISR Varada, which exhibited appreciable growth and rhizome parameters were the high yielders. The genotypes which exhibited higher yield were also good in morphological characters such as plant height, number of tillers, number of leaves, leaf width, leaf area, shoot diameter and rhizome thickness, which helped in assimilating more photosynthates and eventually led to higher yield.

Whereas, the genotype Arunachal local did not record good growth and yield parameters such as plant height, shoot diameter, number of leaves on main shoot, total number of leaves, leaf width and rhizome thickness and yield (4.57 t ha^{-1}).

During 2016/17, highest dry recovery was observed in Acc. 578 and during 2017/18 it was highest in Nadia. Pooled mean analysis showed that, among the genotypes, Acc. 578 recorded higher dry recovery and the genotypes KAU Chandra, Nadia, Gorubathane, Athira, Sourabh, Mohini, Suravi, Suruchi, Karthika, Himachal, RG 3 were on par. Nybe *et al.* (1980) stated that, the variation in drying percentage might be attributed to the difference in size of the rhizome, moisture and fibre content of the cultivars.

5.1.2 Coefficient of variability, heritability and genetic advance as per cent mean

Genetic variability is a prerequisite for response to selection and knowledge of the extent and nature of phenotypic variability is one of the basic needs of the plant breeders for further genetic improvement (Adam, 2006). Selection based on the traits with high PCV and GCV is effective and phenotypic expression of such characters is a good indication of the genotypic potential. Selection of superior genotypes depends on genetic variability existing in the population and selection is more effective when the genetic variation is higher than environmental variation (Allard, 1960).

The knowledge of heritability of traits as they are controlled by genetics and environment and their interaction of these factors become more critical in breeding. The nature and magnitude of variability among the genetic stock of a crop is of prime importance for any crop improvement programmes. Evaluation of variability is important to know the source of gene for a particular trait within the available germplasm (Tomooka, 1991).

The results of the present study revealed that, phenotypic coefficient of variation (PCV) was slightly higher or almost equal to their respective genotypic coefficient of variation (GCV) for all the components studied, but with minimal differences between them, which signifies the presence of environmental influence to some degree. Thus, showing these traits are less influenced by environmental effect which was also reported by Islam *et al.* (2008) and Korla and Tiwari (1999). High heritability generally enables the breeder to select plants on the basis of the phenotypic expression (Johnson *et al.*, 1955). The estimates of PCV ranged from 11.88 % (shoot diameter) to 57.81% (essential oil) and the estimates of GCV varied from 7.25 % (leaf width) to 55.99 % (essential oil). Pandey and Dobhal (1993), Medhi *et al.* (2007), Aragaw *et al.* (2011) also reported higher PCV than GCV in ginger.

In this study close relationship was observed between GCV and PCV revealing very little influence of environment on this trait, which is also reported by Medhi *et al.* (2007). Coefficient of variability varied from character to character (low to moderate or high). This indicates that there is great variability in the experimental material (Mehra, 2012).

During 2016/17 and 2017/18 the parameters such as, total number of leaves, yield per plant and projected yield exhibited high GCV as well as PCV values.

From the pooled data analysis it was observed that, among the characters studied, GCV was found to be high for number of tillers (33.57 %), total number of leaves (23.30 %), yield per plant (32.68 %), essential oil (55.99 %), oleoresin (44.31 %) and crude fibre (45.63 %). Also, similar trend was observed for PCV (number of tillers - 38.82 %, total number of leaves - 28.94 %, yield per plant - 34.93 %, essential oil - 57.81 %, oleoresin - 45.86 % and crude fibre - 46.30 %). The GCV helps in comparison and measurement of genetic variability among the components.

Heritability is the transmissible ability of the characteristics from parent to offspring. The genotypic coefficient of variation does not offer full scope to estimate the variation that is heritable or environmental and therefore, estimation of heritability becomes necessary. In the present study, rhizome thickness exhibited high heritability. Although estimates of heritability were useful to plant breeders as they provide basis for selection, high heritability coupled with high genetic advance as per cent mean will serve as a potential tool for direct selection.

During 2016/17, the parameters such as number of tillers per clump, total number of leaves, yield per plant, as well as projected yield showed high heritability coupled with high GAM. In 2017/18, Number of tillers per clump, number of leaves on main shoot, shoot diameter, rhizome thickness, yield per plant showed high heritability coupled with high GAM.

In the present investigation, pooled analysis exhibited that number of tillers per clump, total number of leaves, yield per plant, essential oil content, oleoresin content and crude fibre exhibited high heritability coupled with high GAM. Hence, selection can be done for these traits in a crop improvement program. Similar findings were reported by Ravishanker *et al.* (2015) for the traits like oleoresin, ascorbic acid, TSS per cent and yield per plant representing additive genetic variance in ginger. These results are in concordance with the findings of Parmar (2011) and Lakshmi and Rajasekhar (2013) in ginger. High genetic gain for number of secondary rhizomes was reported by Mohanty and Sarma (1979), Yadav (1999) and Islam *et al.* (2008); for leaf number by Ali *et al.* (1994), Islam *et al.* (2008) and Rai *et al.* (2008).

5.1.3 Correlation studies

To understand the association of plant characters with yield and to get clear picture of genetic variability, correlation studies are helpful. Correlation analysis followed by the path co-efficient analysis provides a true picture of genetic association among different traits (Bhatt, 1973). Correlation in combination with the path co-efficient analysis quantifies the direct and indirect contribution of one character upon another (Dewey and Lu, 1959).

Phenotypic correlation coefficients showed that, yield per plant had significant and positive correlation with plant height, number of leaves on main shoot, shoot diameter, rhizome thickness and dry recovery. Plant height showed highly significant positive correlation with number of leaves on main shoot, shoot diameter, rhizome thickness and significant correlation with leaf length. Number of tillers showed highly significant and positive correlation with total number of leaves. Number of leaves on main shoot had a highly significant correlation with shoot diameter. Leaf area had highly significant and positive correlation with leaf length and leaf width. These findings are supported by Saikia and Shadeque (1992), Sasikumar *et al.* (1992), Pandey and Dobhal (1993), Abraham and Latha (2003) where the vegetative parameters *viz.,* plant height, number of leaves, shoot diameter and rhizome character like rhizome thickness showed positive correlation with yield. Nandkangre *et al.* (2016) also reported high significant correlations among rhizome yield, rhizome weight per plant, numbers of leaves, leaf length and plant height.

Essential oil has shown highly significant and positive correlation with oleoresin and crude fibre. Whereas, the rhizome thickness exhibited significant and negative correlation with essential oil, oleoresin and crude fibre. This might be due to, less number of total oil cells when the thickness of the rhizome increases. Also, bold rhizomes recorded less crude fibre content and are suitable for vegetable purpose.

The genotypic correlation coefficients between different characters revealed that, yield per plant had highly significant and positive correlation with plant height, number of leaves on main shoot, shoot diameter, rhizome thickness, dry recovery and significant positive correlation with leaf length. Similarly like phenotypic correlation, genotypic correlation for plant height showed positive association with the number of leaves on main shoot, shoot diameter and rhizome thickness. Number of tillers showed a highly significant positive correlation with total number of leaves. Number of leaves on main shoot exhibited positive correlation with leaf length, shoot diameter and rhizome thickness. Similar findings have been reported by Ravishanker *et al.* (2013), where the rhizome yield had significant positive correlation with plant height, length of primary rhizome and rhizome thickness in ginger. The characters that displayed consistency in trait association over the years could be the potential traits that need to be addressed for the improvement of gingers. Plant height appeared to display significant and positive correlation with yield contributing traits. The same trait was also concluded to be of prime importance in the selection program on the basis of its positive and significant correlation with rhizome yield (Sasikumar *et al*., 1992).

5.1.4 Grouping of genotypes as per DUS guidelines

The candidate varieties for DUS testing shall be divided into groups to facilitate the assessment of Distinctiveness. Characteristics, which are known from experience not to vary, or to vary only slightly within a variety and which in their various states are fairly evenly distributed across all varieties in the collection are suitable for grouping purposes (PPV&FRA, 2007).

Figure 6 shows the summary of grouping of genotypes as per the DUS guidelines in the present investigation. Ginger genotypes were grouped as per the DUS guidelines of ginger whereas black ginger and mango ginger were grouped as per turmeric guidelines.

Growth habit of the 30 genotypes was found to be polymorphic with 16 genotypes (53.33 %) having erect growth habit, 2 genotypes (44.45 %) exhibited semi erect growth habit and 2 genotypes (black ginger and mango ginger) exhibited open (6.66 %) growth habit. Plant height was a dimorphic character with 29 genotypes (96.66 %) were short and one genotype *i.e.* mango ginger (3.33 %) was tall. Number of tillers was found to be polymorphic characters which were grouped as 20 genotypes (66.66 %) with few tillers, eight genotypes (26.66 %) with medium (10-15) tillers and two genotypes (6.66 %) with many tillers. Twenty six genotypes (96.3 %) exhibited narrow (<3 cm) shoot diameter and one genotype (3.7 %) Aswathy exhibited medium (3-5 cm) shoot diameter. Similar reports were reported by Aswathy (2013) in the characterization of ginger somaclones where the plants were erect or semi erect and majority of the somaclones were short. Somaclones exhibited fewer numbers of shoots and narrow sized pseudostem.

Leaf characters under study as per the DUS guidelines were number of leaves on main shoot, leaf length and leaf width. All the three characters were found to be dimorphic. Twenty nine (96.66 %) genotypes exhibited few numbers of leaves on main shoot and one genotype (3.33 %) *i.e.,* mango ginger exhibited intermediate number of leaves on main shoot. In case of leaf length twenty nine genotypes (96.66 %) were having short leaf length and one genotypes (3.33 %) mango ginger was having long leaf length. Leaf width was dimorphic character with narrow and medium leaves. Twenty eight genotypes (93.33 %) exhibited narrow leaf width and the variation was in mango ginger and black ginger (6.66 %) which exhibited medium leaf width. Results are in accordance with that of Aswathy (2013) where the leaves of ginger somaclones were fewer, short and narrow.

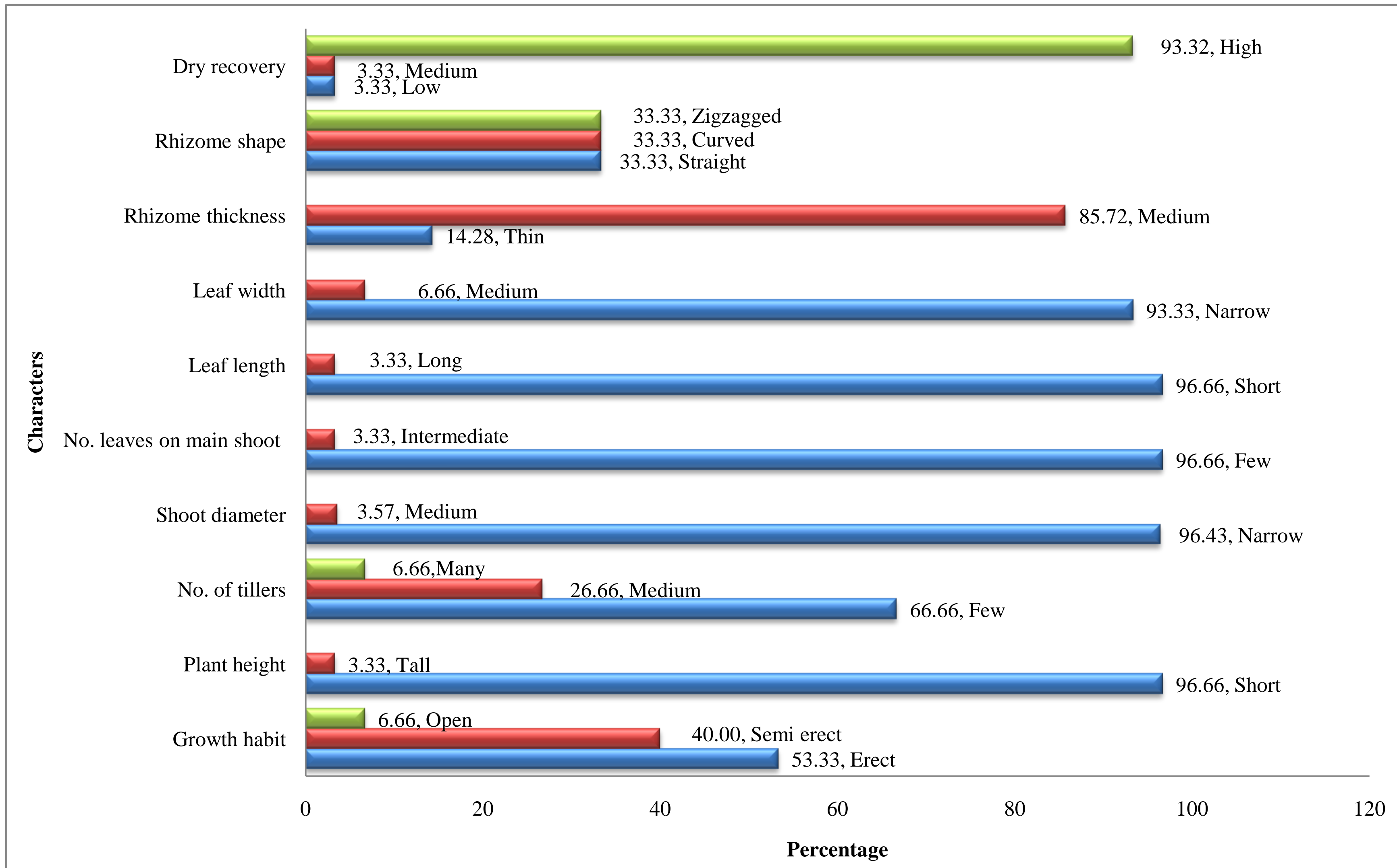


Fig. 6: Grouping of ginger genotypes as per the DUS guidelines (PPVFRA, 2007)

Rhizome being the economic part in ginger these characters plays an important role in differentiating the genotypes into different categories and which ultimately influence the yield. Rhizome thickness exhibits positive correlation with rhizome yield. Ravishanker *et al.* (2013) also reported similar findings where rhizome yield had significant positive correlation with rhizome thickness. Rhizome thickness was dimorphic character and among the 28 ginger genotypes, four genotypes exhibited <2 cm (thin) rhizome thickness where the rhizomes were slender and 23 genotypes exhibited 2-3 cm (medium) rhizome thickness. Rhizome shape was found to be polymorphic character and there was equal distribution of genotypes *i.e.,* ten genotypes (33.33 %) in each category (straight, curved and zigzagged).

Yield per plant on dry weight basis is dependent on the dry recovery (%). Higher the dry recovery more will be the yield. Among the 30 genotypes, red ginger (3.33 %) exhibited low dry recovery of > 16 %, one genotype (3.7 %) Rio de Janeiro recorded medium (16-18 %) dry recovery and other 28 genotypes (93.32 %) exhibited high (>18 %) dry recovery. Nybe *et al.* (1980) stated that, the variation in drying percentage might be attributed to the difference in size of the rhizome, moisture and fibre content of the cultivars.

Results observed in case of rhizome characters in the present study were in accordance with the results obtained by Aswathy (2013), where all the three types of rhizome shapes were observed and rhizomes were of medium size in ginger somaclones.

From this grouping of genotypes as per the DUS guidelines it can be concluded that the difference in the morphological characters among the genotypes was narrow and most of the genotypes were grouped together in each category.

5.2 Development of chemical profiles

Biochemical parameters which ultimately decide the quality of ginger are essential oil, oleoresin and crude fibre. These quality characters are of great importance in selecting the genotype/variety for different end use.

5.2.1 Oleoresin

Flavour and pungency of ginger is valued by the quantum of oleoresin present in the rhizomes (Menon, 2007). Organoleptic properties of the ginger oleoresin are the principal factors responsible for the flavour of processed food, pharmaceutical preparation and beverages. Oleoresin yield of ginger usually varies from 3 to 11% (Vermin and Parkanyi, 2005).

In the present study, pooled data as well as year wise data exhibited that Red ginger had the highest oleoresin percentage of 12.18 % and whereas lowest was in Acc. 578 (3.28 % during 2016/17; 2.94 % during 2017/18 and 3.11 % pooled value). Among other ginger genotypes, Arunachal Pradesh local (8.55 %), Rio de Janeiro (7.77 %) and Acc. 65 (7.10 %) recorded high oleoresin content. The high oleoresin content obtained in red ginger is in contradiction with the results of Kusumawati *et al.* (2017) where the oleoresin content of red ginger of Indonesia was 0.88 %. The discrepancies among the above studies show that variations in geographical regions affect the oleoresin content of ginger.

Genotypes, harvesting stage, cultivation practices, choice of solvents and method of extraction *etc.* are known to affect the oleoresin content in ginger (Connel, 1969). In the present study, all the factors except genotypes being common, the variability observed for oleoresin may be attributed to the effect of the cultivars. The cultivars (Arunachal Pradesh local, Rio de Janeiro and Acc. 65) rich in oleoresin are the better sources for commercial exploitation for oleoresin extraction.

5.2.2 Crude fibre

Fiber content is the most important criteria for assessing the suitability of ginger rhizome for specific products like ginger paste, salted ginger, ginger powder *etc.* The processed foods such as jam, marmalade, cake and confectionery industries prefer less fiber gingers.

Fiber content in ginger is highly affected by the factors such as soil type, cultural practices, analytical method, genotypes *etc.* (Ajithkumar and Jayachandran, 2003; Tiwari, 2003 and Yiljep *et al.,* 2005).

The crude fibre content of unpeeled ginger may be as high as 10% (on a dry weight basis), but in commercial dried gingers it is usually in the range of 1.5–6% (Zacharia, 2008).

Both low fiber and high fiber gingers are important depending on the end use. During 2016/17 and 2017/18 both the years lowest fibre content was observed in Acc. 578 followed by Nadia and highest fibre content was recorded in red ginger. In the present study cultivars such as, Nadia and Acc. 578 which recorded very less fiber and hence can be exploited for making varied value added products like slices preserved in syrup, ginger candy (crystallized ginger), jams, cakes *etc.* Whereas, the cultivars Red ginger, Acc. 219, and Arunachal Pradesh local which recorded high fiber content is useful for dry ginger industry.

5.2.3 Essential oil

Yield of essential oil in ginger ranges from 0.2 to 3.0%, depending on the origin and state of the rhizome (Ekundayo *et al*., 1988 and Van Beek *et al*., 1987). The volatile oil content of commercial dried gingers has been reported to be 0.5–4.4% but, for the major types, the range is usually 1–3% (Zacharia, 2008). In the present study also among the ginger genotypes highest oil content of 3.00 % was recorded in Arunachal Pradesh local. Similar results were obtained by Jaleel and Sasikumar (2009) where the primitive ginger types and land races like pink ginger, Sabarimala, Kozhikkalan and Kakakalan were reported with higher oil content than the improved varieties. Chongtham *et al.* (2013) also reported that the local germplasm were better following standard package of practices. The improved cultivars possibly could not exhibit their fullest potential due to variation in soil and climatic conditions from the area of collection.

Whereas 6.0 % oil content was recorded in the variant of normal ginger, Red ginger *i.e., Zingiber officinal* var. *rubra*. In earlier report also the oil content of red ginger was reported to be high 3.9 % (Nwinuka, 2005), 2.11 % (Kusumawati *et al.* 2017).

Along with 28 ginger genotypes two other related gingers were also used for essential oil estimation for comparison purpose *viz.,* black ginger (*Kaempferia parviflora*) and mango ginger (*Curcuma amada* L.) where the essential oil content was 0.1 % and 1.0 %, respectively.

5.2.4 Essential oil yield per unit area

Essential oil yield per unit area gives the actual picture of the total availability of oil per unit area based on the crop yield per unit area and the essential oil percentage of each genotype. In case of twenty eight ginger genotypes studied highest essential oil yield per hectare of 106.94 l/ha was obtained in the genotype Maran which exhibited highest rhizome yield. In case of red ginger which was having highest percentage of essential oil (6 %) exhibited essential oil yield per unit area of 58.53 l/ha, this difference is due to the less yield of red ginger and same in the case of the genotype Arunachal Pradesh local which was having the highest oil percentage of 3 % where the rhizome yield was less. The genotypes which are having higher oil percentage with less rhizome yield can be improved by adopting improved agronomic practices or by adopting crop improvement technique *i.e.,* polyploidy breeding.

5.2.5 GC/MS analysis of essential oil

Essential oil contains compounds which give pleasant aroma to ginger. Thus, analysis of essential oil composition is indispensable in determining the various grades and prices of the produce. Mono and sesquiterpenoids are present in the volatiles, but sesquiterpenoids are quantitatively the major constituents (Ekundayo *et al*., 1988).

Thirty five essential oil components were identified in the present investigation, using 28 ginger genotypes along with mango ginger and black ginger. More number of compounds was observed in Aswathy, Suprabha and Mohini around 28 compounds and less number (13) of compounds in black ginger.

The constituents identified in ginger oil were grouped into different groups such as sesquiterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene alcohols and monoterpene hydrocarbons. Sesquiterpene hydrocarbons are beta-Elemene, beta.-farnesene, zingiberene, beta-bisabolene, alpha-farnesene, alpha-curcumene, beta.-sesquiphellandrene and elemol. Oxygenated monoterpenes were 1, 8 cineole, terpinolene, beta-linalool, beta-citral, alpha-terpineol, borneol, beta-geraniol. Sesquiterpene alcohols were alpha.-eudesma, trans-nerolidol, beta.-eudesmol. Monoterpene hydrocarbons are alpha-pinene, camphene, beta.-pinene, beta.-myrcene, alpha-limonene, beta-phellandrene, alpha-copaene.

In the present study zingiberene was the major component present in the essential oil comprising maximum peak area in all the ginger genotypes analyzed. Higher percentage of zingiberene (33.42 %) was observed in genotype 'Maran' followed by 'Suprabha' (30.16 %) (Fig. 7). The specific aroma of ginger is predominantly related to Zingiberene. Similar results on Zingiberene as the major compound in ginger was reported by earlier workers also (Sultan *et al.,* 2005; Raina *et al.,* 2005; Jaleel and Sasikumar, 2012; Kiran *et al.,* 2013, Toure and Xiaoming, 2007) and in comparison of current findings with previous studies varying percentage of zingiberene in different ginger rhizomes has been reported by Raina *et al*., 2005 (10.5% - 16.6%), Sultan *et al*., 2005 (30.81 %), Jaleel and Sasikumar, 2012 (17.6-29.6 %), Kiran *et al.,* 2013 (20.98 %).

Beta sesquiphellandrene content in the ginger genotypes varied from 8.48 % (Acc. 833) to 21.78 % (Red ginger) (Fig. 8). Onyenekwe and Hashimoto (1999), Miyazawa and Kameoka (1988) also reported presence of beta sesquiphellandrene compound in ginger oil. The content of beta bisabolene ranged from 14.09 % (IISR Varada) to 2.34 % (Sourabh). Percentage of beta bisabolene was found to be highest after zingiberene and beta sesquiphellandrene. Miyazawa and Kameoka (1988) reported 7.9 % of beta bisabolene content in ginger.

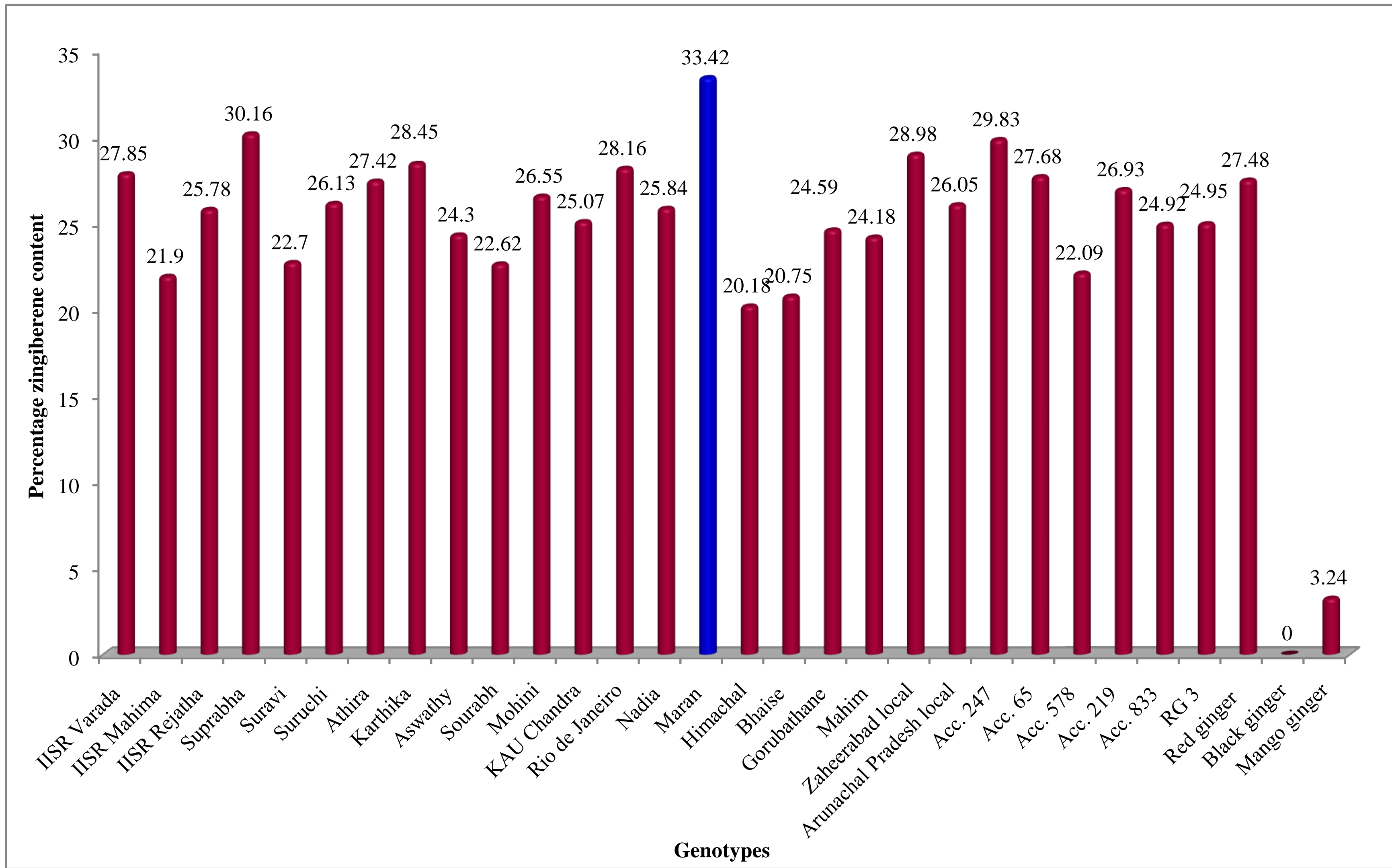


Fig. 7: Percentage zingiberene content in ginger genotypes

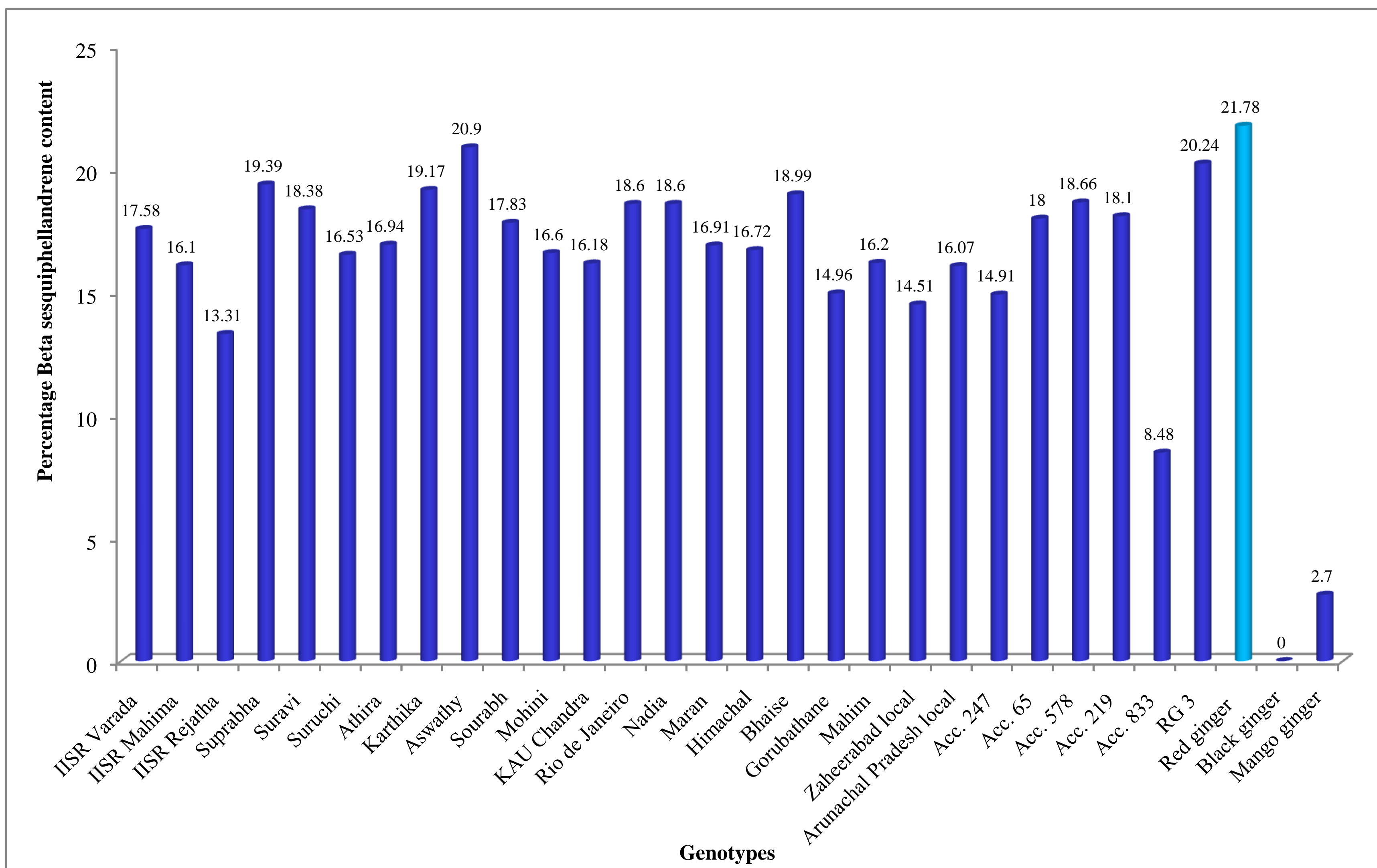


Fig. 8: Percentage beta sesquiphellandrene content in ginger genotypes

Lemony aroma of the ginger is mainly due to citral (geranial + neral) content. Moderate citral content was reported in the essential oils of ginger from different parts of India (Raina et al., 2005). Our results also showed moderate citral content in the essential oil in some of the accessions such as 'Zaheerabad local' (9.69 %), 'IISR Rejatha' (9.51 %), 'Suravi' (7.01 %), 'IISR Mahima' (6.63 %), 'IISR Varada' (6.62 %), 'RG 3' (6.51 %), 'Acc. 65' (6.48 %) and 'Bhaise' (6.11 %). From the results obtained in the present study and in the previous studies it is evident that percentage of volatiles and the composition vary considerably depending on chemo-taxonomic differences between cultivars and region of production and the agro-climate conditions. Similar report was reported by Raina *et al.* (2005).

In case of alpha farnesene, IISR Rejatha recorded maximum content of 15.31 % and was lowest in mango ginger (0.81 %). This compound was not present in black ginger. Purseglove *et al.* (1981) also reported farnesene upto 10 %. Alpha pinene content varied from 0.25 % (Acc. 833) to 13.17 % (black ginger). Sultan *et al.* (2005) reported 3.59 % of this compound in Thailand ginger and in case of China ginger it was 0.305 %. In black ginger, it was found to be one of the major compounds.

Maximum quantity of camphene was observed in black ginger (20.21 %), among ginger genotypes it was maximum in Arunachal Pradesh local (3.95 %) and minimum in Acc. 833 (0.9 %). Camphene was also found to be one of the major compounds in black ginger. Miyazawa and Kameoka (1988) reported 1.4 % of camphene in ginger oil whereas Kiran *et al.* (2013) reported 8.49 % of camphene in the ginger volatile oil from North East.

In mango ginger, the quantity of beta pinene was maximum (15.17 %) and in black ginger it was 2.0 %. In case of ginger genotypes, it was in very less quantity and was absent in nine genotypes. Sultan *et al.* (2005) also reported 0.74 % of this compound in Thailand ginger and Jaleel and Sasikumar (2012) noticed 0.46 % (Mananthodi) to 1.9 % (Kintoki) of this compound. Beta myrcene content varied from 0.35 % (Acc. 833) to 1.06 % (red ginger). It was in less quantity in many of the ginger genotypes, which varied from 0.45 % -1.04 % and it was absent in mango ginger, Karthika, Mahim, Suprabha, RG 3 and Suruchi.

Percentage of alpha limonene content varied from 0.41 % (Acc. 833) to 2.37 % (Gorubathane). Only in few genotypes *viz.,* black ginger (2.26 %), Mahim (1.16 %), red ginger (1.16 %) and Suruchi (1.04 %) the content was more than 1 %. Out of 30 genotypes, beta phellandrene was found to be 1.48 % in Maran and was trace in other genotypes.

Furan was absent in ginger genotypes and present only in mango ginger which covered area percentage of 4.41. Beta linalool is one of the major compounds present in black ginger with area percentage of 23.3%. The content varied from 0.43 % (Karthika) to 1.71 % (Acc. 65) in other genotypes.

In the red ginger oil the major chemical constituents were zingiberene (27.48 %) followed by beta-sesquiphellandrene (21.78 %) (Fig. 9). Sivasothy *et al.* (2011) mapped the volatile oil composition in the leaves and fresh rhizomes of red ginger obtained from Negeri Sembilan, Malaysia. Forty-six compounds were detected in the leaves and 54 compounds in the rhizomes, the main constituents of which were monoterpenoids (81.9%), with the highest being camphene (14.7%), followed by geranial (14.3%), geranyl acetate (13.7%), neral (7.7 %), geraniol (7.3%), and 1.8-cineole (5%). Neral and geranial are suspected to cause the scent of lemon found in red ginger rhizomes. In our study zingiberene was the major compound, this difference between both the studies might be due to variation in the samples and also maturity level of the rhizomes. In our study dried rhizomes were used for oil extraction whereas Sivasothy *et al.* (2011) used fresh rhizomes. Our study is the first report on the chemical composition of red ginger oil in India.

In case of black ginger the number of chemical compounds detected was less as compared to other ginger genotypes. Number of compounds detected was only 13 (Fig. 10). Beta linalool (23.30 %) followed by camphene (20.21 %), germacrene D (16.00 %)

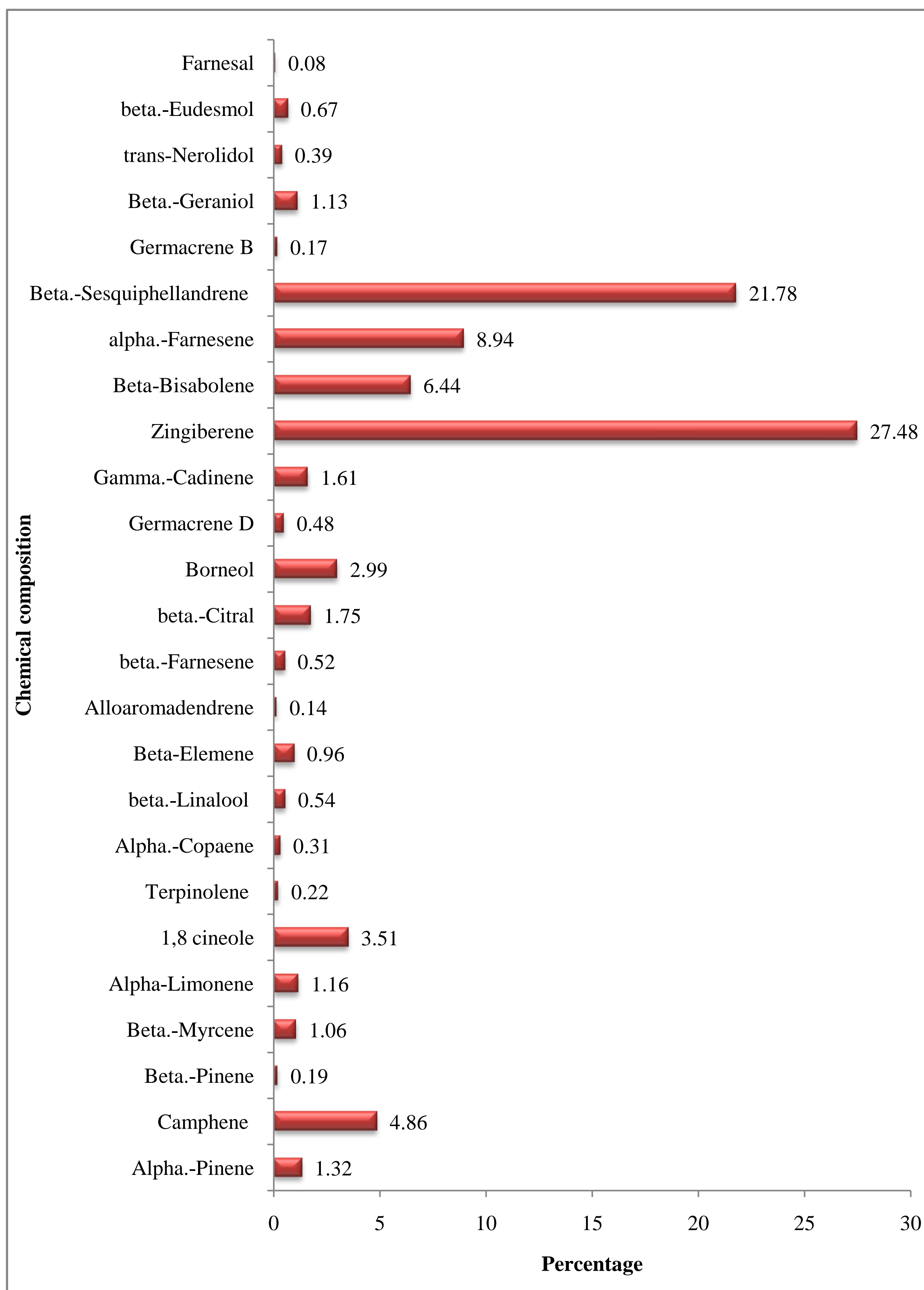


Fig. 9: Chemical profile of red ginger

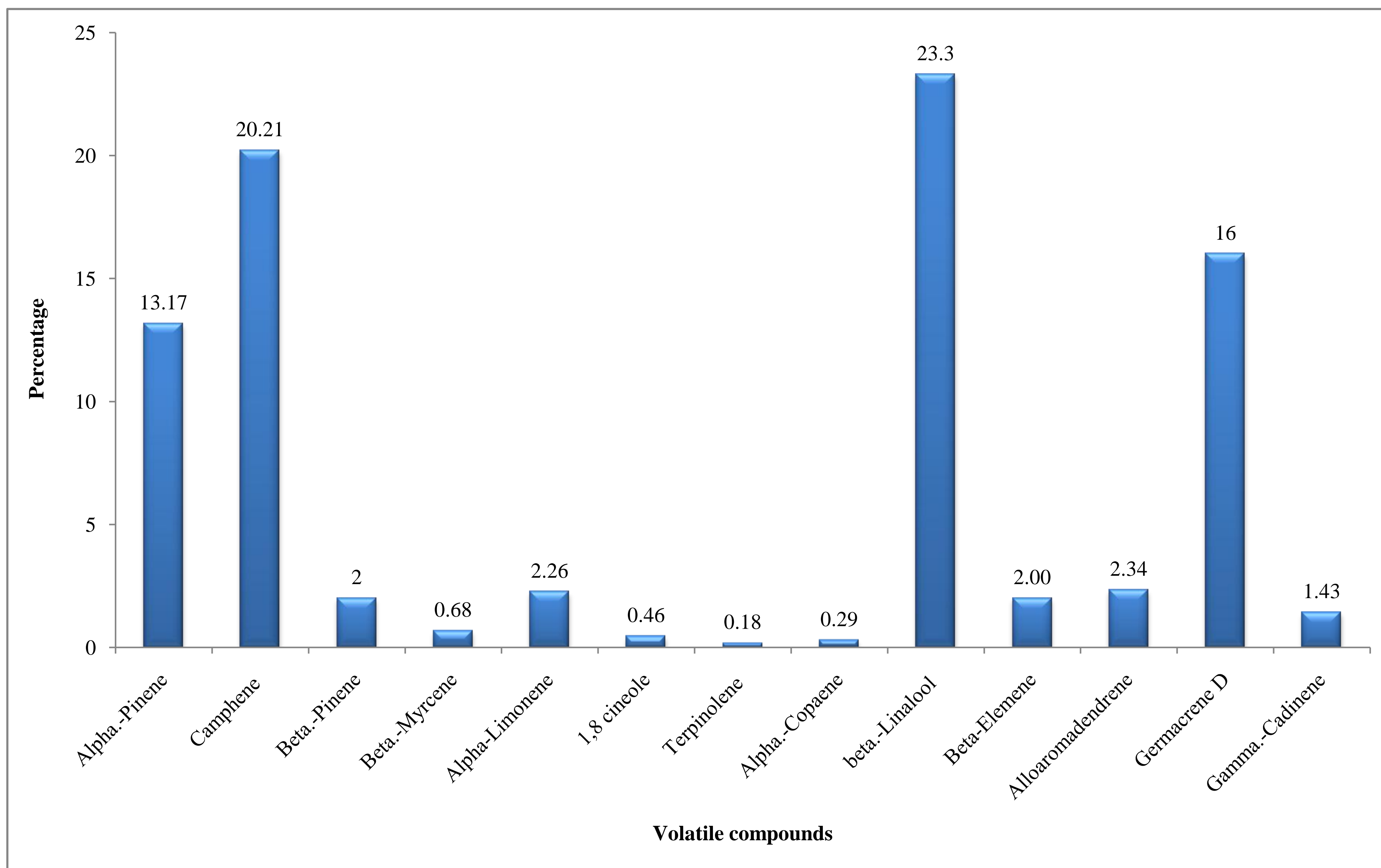


Fig. 10: Chemical profile of black ginger

and alpha pinene (13.17 %) were the major compounds. This is also the first report on the chemical composition of black ginger identified by GC/MS analysis.

5.3 Molecular characterization

Knowledge of the genetic variation within and among populations is an important component for understanding the variability in any crop. Therefore, information on population diversity may be used in selection and crop improvement process. Molecular methods are much faster, more specific, sensitive and accurate. Molecular markers are nowadays widely used to distinguish the genotypes in several horticulture crops (Li *et al.,* 2007; Karimi *et al.,* 2010 and Ansari and Singh, 2013 & 2014).

As ginger is clonally propagated and it is difficult to distinguish between the genotypes using morphological markers, molecular approaches are highly useful for characterization of ginger genotypes. In the present study 30 RAPD and 55 SSR markers were used to study the genetic variability.

5.3.1 Characterization of ginger genotypes using RAPD markers

In the present study, the coefficient of genetic similarity ranged from 39 - 97 per cent. Two related genotypes *i.e.,* mango ginger and black ginger which were used in the study were out grouped from other ginger genotypes in group I with similarity percentage of 39. This clearly shows that the RAPD primers used were highly efficient in separating the species other than *Zingiber.* Other 28 ginger genotypes were grouped together in group II, which showed that there is similarity in the genotypes under study. Among the ginger genotypes, higher percentage of similarity was observed between genotypes Zaheerabad local and Himachal (97 %). The genotype Arunachal Pradesh local collected from Arunachal Pradesh clustered separately which showed that, it may be due to the geographical origin of the genotype. Similar trend was not observed in case of all the genotypes under study, clustering was not according to the geographical origin.

RAPD dendrogram was not associated with exact geographical localities from which the ginger genotypes were collected. The considerable polymorphism detected in this study illustrated that, it is possible to find genetic divergence among ginger cultivars of the same origin. These results are in accordance with Nayak *et al.* (2005) and Sera *et al.* (2003), who also reported similar results in ginger and coffee respectively.

These results in ginger indicate that, RAPD markers were able to provide more reliable information than morphological characters to identify closely related ginger genotypes (Nayak *et al.,* 2005 and Palai and Rout, 2007). Diversity among the cultivars revealed the presence of genotypic diversity among the genotypes. Variability to certain extent might be due to the different environmental conditions.

5.3.2 Characterization of ginger genotypes using SSR markers

SSRs are widely used as versatile tool in plant breeding programme as well as in evolutionary studies because of their ability for showing diversity among the cultivars (Adato *et al.,* 1995). Therefore, in the present investigation, out of 55 SSR primers screened, 16 primers amplified and produced 34 alleles among them 23 were polymorphic bands and 11 were monomorphic bands. Pandotra *et al.* (2013); Das *et al.* (2016); Jatoi *et al.*, (2006) and Lee *et al.* (2006) also reported the use of SSR markers to study the variability and genetic diversity existing at the population level.

Dendrogram obtained revealed that, irrespective of their place of collection or geographical origin they have grouped into different clusters which showed that, each genotype selected in the study is having wide variability or it may be due to genetic similarity existing among them.

Phylogenetic analyses of 30 genotypes, conducted on SSR banding patterns, indicated that, maximum percentage of similarity (100 %) was observed between KAU Chandra, IISR Mahima and Mohini; IISR Rejatha and Nadia; Acc 65, Suprabha and Maran; Rio de Janeiro and Sourabh; Suruchi and Acc. 833; IISR Varada and Bhaise. Two genotypes other than *Zingiber sp. i.e.,* mango ginger and black ginger, which were used in the study, mango ginger was out grouped from other ginger genotypes whereas black ginger

was grouped with one of the ginger genotype Acc. 578. This clearly shows that the SSR primers used were highly efficient in separating *Curcuma* sp. from the *Zingiber* species but those didn't distinguish the ginger genotypes based on any character or place of collection. Jatoi *et al.* (2006) also reported that clustering pattern within the genus *Zingiber* did not reflect any relationship between genotypic variation and place of collection.

5.3.3 Molecular variability of ginger genotypes through pooled RAPD and SSR markers

Data obtained from pooled analysis of RAPD and SSR primers revealed that the ginger genotypes were divided into 2 main groups I and II sharing 49 % similarity. From the pooled analysis of RAPD and SSR markers also it was clear that the markers were efficient in clustering the other species mango ginger and black ginger but in case of ginger genotypes dendrogram revealed that irrespective of place of collection or origin genotypes were grouped into different clusters. Grouping was not on the basis of any morphological, yield or biochemical characters. Similar results were obtained by Jaleel and Sasikumar (2010) and they reported that, collection of the accessions based vernacular identity irrespective of the geographical proximity may be the probable reason for this behaviour. It also implies that genes amplified by the markers need not be strictly linked with any agronomic traits.

5.3.4 Identification of Single Nucleotide Polymorphism

Identification of SNPs using comparative transcriptome was carried out which can be further utilized to identify the genotype specific markers. SNPs are less mutable as compared to other markers particularly microsatellites. The low rates of recurrent mutations make them evolutionarily stable. They are excellent markers for studying the complex genetic traits and for understanding the genomic evolution (Jehan and Lakhanpaul, 2006). Therefore, in this study SNP identification was carried out by using five different transcriptome sequences.

IISR Varada was used as reference transcriptome sequence and other four other genotype transcriptomes were compared with it to identify the SNPs. Sixty one thousand four hundred and twenty four common transcripts were obtained when the transcriptome of Queensland and IISR Varada transcriptomes compared, out of which 33,694 SNPs were identified in coding sequences and 27,730 SNPs in non coding sequences. Among the SNPs in the coding sequences, 19,268 were synonymous and 14,426 were non synonymous. Similarly total of 43,635 similar transcripts were detected in the comparison of Maran and IISR Varada transcriptomes, out of which 27,863 SNPs were in coding sequence and 15,772 SNPs were in non-coding sequences. Out of 27,863 SNPs obtained, 16,657 SNPs were synonymous and 11,206 SNPs were non synonymous.

Total number of similar transcripts obtained by comparison of Yujiang 1 and IISR Varada transcriptomes were 59,081 and out of which 33,011 SNPs in CDS and 26,070 SNPs in non coding sequences. Out of 33,011 SNPs in CDS, 19,261 SNPs were synonymous and 13,750 were non synonymous. In case of comparison of transcriptomes of Southwest and IISR Varada a total of 56,194 similar transcripts were detected, out of which 31,983 SNPs were in coding sequence and 24,211 SNPs were not in non coding regions. Out of 31,983 SNPs obtained in CDS, 18,678 SNPs were synonymous and 13305 SNPs were non synonymous. Similar studies were also carried out by He *et al.* (2017) in *Pheobe chekiangensis*; Farrell *et al.* (2014) in perennial ryegrass where the reference genome is not available.

Venn diagrams representing comparison of the number of SNPs among the different transcriptomes is presented in Fig. 11. This shows the number of common SNPs between the genotypes. 2957 SNPs were specific for Queensland and IISR Varada; 119 SNPs for Maran and IISR Varada transcriptomes; 1321 SNPs for Yujiang 1 and IISR Varada transcriptomes and 999 SNPs for Southwest and IISR Varada. Whereas 17,630 SNPs were common among all the five genotypes.

A large amount of SNP markers boosted comparative transcriptome approaches to identify novel SNPs genotyping. Nevertheless, no SNP resources have been previously reported for ginger. The high percentages of non-synonymous SNPs indicating that the functional mutation rate was high.

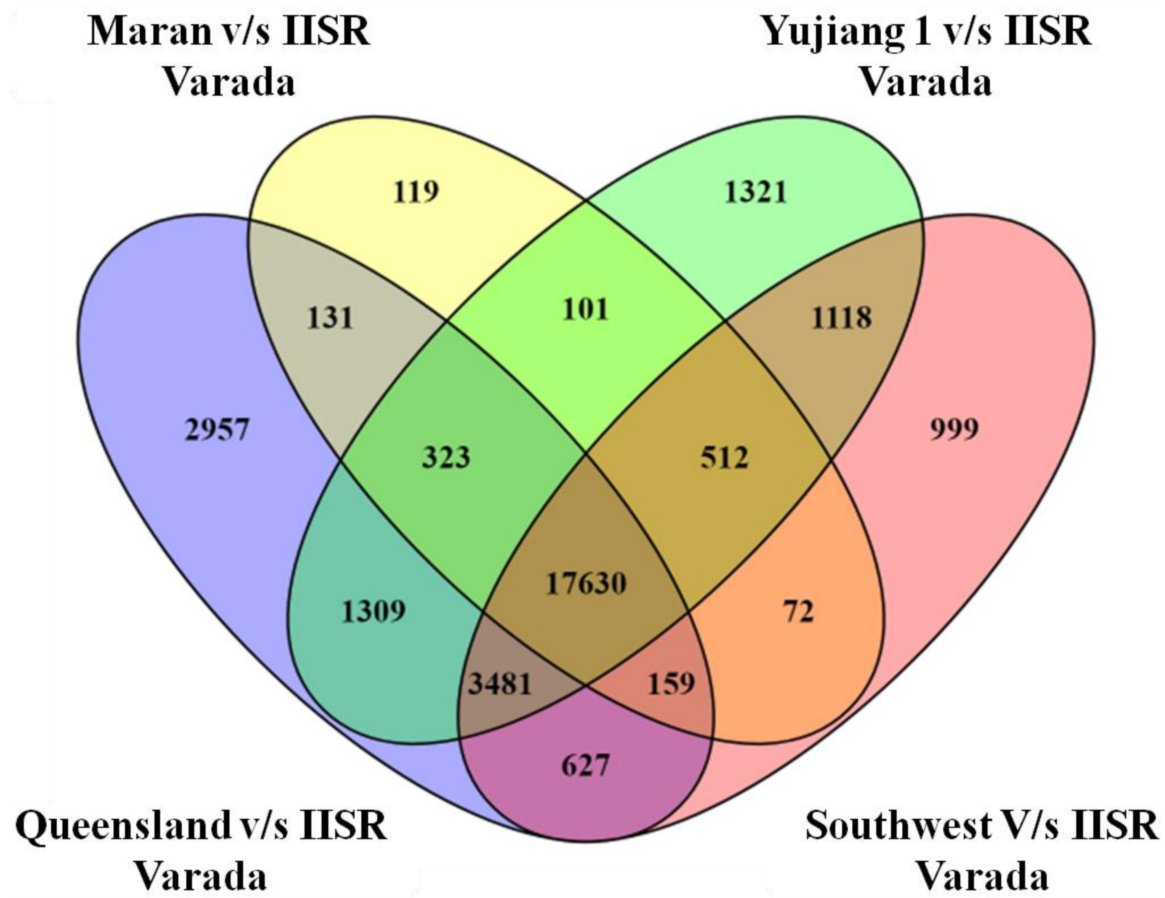


Fig. 11: Venn diagram representing comparison of the number of SNPs among the different transcriptomes

VI. SUMMARY

Studies on “Molecular and chemo profiling of ginger (*Zingiber officinale* Rosc.) genotypes” were carried out during 2016-17 and 2017-18 at ICAR-Indian Institute of Spices Research, Kozhikode, Kerala and ICAR-Indian Institute of Spices Research, Experimental Farm, Peruvannamuzhi, Kerala.

The study was conducted to know the morphological, biochemical and molecular diversity among the ginger genotypes. Twenty eight ginger genotypes, one *Curcuma* sp. and one *Kaempferia* sp. were used for the study. The salient findings of the study are summarized below.

Significant differences were observed among the genotypes for all the characters *viz.,* plant height, number of tillers, number of leaves on main shoot, total number of leaves, leaf width, leaf area, shoot diameter, rhizome thickness and yield parameters except for leaf length.

In case of morphological characterization, the genotype Maran recorded the maximum plant height of 73.01 cm, more number of tillers per clump (15.25) in Mahim, more shoot diameter of 3.12 cm in Aswathy, more number of leaves on main shoot in IISR Varada (16.90), and more number of total leaves per clump in Suravi (127.45). Genotype RG 3 recorded maximum leaf width (2.35 cm) as well as leaf area of 33.31 cm^2. The maximum rhizome thickness was observed in cultivar Bhaise (2.55 cm), whereas, the highest projected yield was recorded in genotype Maran (17.71 t ha^{-1}) followed by Acc. 247 (16.33 t ha^{-1}), Himachal (16.06 t ha^{-1}) and IISR Varada (15.85 t ha^{-1}) and the dry recovery was highest in Acc. 578 (26.13 %).

The results revealed that, phenotypic coefficient of variation (PCV) was slightly higher or almost equal to their respective genotypic coefficient of variation (GCV) for all the components studied, but with minimal differences between them, which signifies the environmental influence to some degree. The estimates of PCV ranged from 11.88 % (shoot diameter) to 57.81% (Essential oil) and the estimates of GCV varied from 7.25 % (leaf width) to 55.99 % (essential oil). Number of tillers per clump, total number of leaves, yield per plant, essential oil, oleoresin and crude fibre content exhibited high heritability coupled with high GAM. Hence, these traits can be employed in a crop improvement program.

Phenotypic correlation coefficients showed that yield per plant had significant and positive correlation with plant height, number of leaves on main shoot, shoot diameter, rhizome thickness and dry recovery. Essential oil showed highly significant and positive correlation with oleoresin and crude fibre. The genotypic correlation coefficients between different characters revealed that, yield per plant had highly significant and positive correlation with plant height, number of leaves on main shoot, leaf length, shoot diameter, rhizome thickness and dry recovery.

Ginger genotypes were grouped as per the DUS guidelines by using quantitative and qualitative traits. From this grouping of genotypes it can be concluded that the difference in the morphological characters among the genotypes was narrow and most of the genotypes were grouped together in each category. Grouping of genotypes with respect to the rhizome characters gives an insight in to the availability of variation among the genotypes and using the genotypes with good rhizome characters for further selection and crop improvement programmes as well as protection of plant varieties.

For chemical profiling the genotypes included in the present study consisted of released varieties, local cultivars and promising genotypes from germplasm collection. Many of the released varieties and local cultivars are under cultivation in different parts of India. Present investigation gives an insight into quality and chemical composition of the genotypes which assist in selection of genotypes for different end use.

Red ginger had the highest oleoresin as well as essential oil percentage of 12.18 % and 6.00 % respectively. Among other ginger genotypes evaluated, Arunachal Pradesh local (8.55 %), Rio de Janeiro (7.77 %) and Acc. 65 (7.10 %) revealed high oleoresin content and genotype Arunachal Pradesh local had higher oil content of 3.00 %. In the present study, cultivars such as, Nadia, Acc. 578 and Aswathy recorded very less fiber and hence can be exploited for making value added products like slices preserved in syrup, ginger candy (crystallised ginger), jams, cakes *etc.* Whereas, the cultivars such as Red ginger, Acc. 219, Acc. 833 and Arunachal Pradesh local with high fiber content will be useful for dry ginger making. Estimation of essential oil per unit area gave an insight into the actual yield of essential oil per unt area based on the yield

parameters. Zingiberene was the major component present in the essential oil of ginger genotypes, beta linalool was the major compound in black ginger, whereas in mango ginger major compound was beta pinene.. The cultivar Maran with highest percentage of zingiberene is useful for bioprospecting. This is the first report on the GC/MS profiling of red ginger from India and also black ginger.

In molecular profiling, out of 11 RAPD primers used, ten primers *viz.*, OPA 09, OPA 17, OPA 18, OPB 08, OPD 03, OPD 07, OPD 18, OPH 08, OPI 07 and OPL 12 were found to show 100 per cent polymorphism and the Polymorphic Information Content (PIC) was high in OPD 03, OPD 07 and OPH 08 (0.998). In case of SSR primers, maximum number of alleles detected was seven from ZOM 103 primer. With the average of 62.80 per cent polymorphism produced by sixteen SSR primers, cent per cent polymorphism was detected by the primers ZOC 11, ZOC 28, ZOC 156, ZOC 33, ZOM 064, ZOM 140 and CLEST 16.

Irrespective of their place of collection or geographical origin, 30 genotypes were clustered into different groups which showed that, each individual genotype is having wider variability or it may be due to the genetic similarity existing among them.

Identification of SNPs was carried out which can be further utilized to identify the genotypes. In the comparison of Queensland and IISR Varada transcriptomes 2957 SNPs were specific for these two transcriptomes and for Maran and IISR Varada transcriptomes 119 SNPs were specific. For Yujiang 1 and IISR Varada transcriptomes 1321 SNPs were specific and for Southwest and IISR Varada 999 SNPs were specific. Whereas, 17,630 SNPs were common among all the five transcriptomes.

Conclusion

Growth and yield parameters such as number of tillers per clump, total number of leaves, yield per plant, essential oil, oleoresin and crude fibre content exhibited high heritability coupled with high GAM are reliable selection parameters for ginger crop improvement. The ginger genotypes such as red ginger and Arunachal Pradesh local for high oil and oleoresin; red ginger, Acc. 219 and Arunachal Pradesh local for high fibre content and Nadia, Acc. 578 and Aswathy for low fibre content have been identified. Single Nucleotide Polymorphism (SNPs) have been identified which would be of immense use in future genetic diversity studies in ginger.

Future line of work

- The study can be extended for screening other available ginger germplasm for assessing their genetic diversity to identify the genetically diverse types for future crop improvement and to avoid the redundancy/duplicates.
- SNPs identified in the present investigation need to be further characterized and validated for genetic diversity studies in ginger.
- Red ginger was superior by possessing high oil and oleoresin content with almost similar chemical profiles as that of other ginger genotypes has low yield potential which need to be improved by different crop improvement techniques.
- The present study need to be continued for assessing genetic diversity of other ginger genotypes, accessions and other related species of genus *Zingiber* to exploit full genetic potential available in this commercial crop.

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*Originals not found

APPENDIX I

Meteorological data of ICAR-IISR Experimental Farm, Peruvannamuzhi during the experimental period

Month	Year	Temperature (°C)		Relative humidity (%)		Rainfall (mm)	Rainy days	Sunshine hours
		Max.	Min.	FN	AN			
June	2016	29.40	22.91	94.80	86.00	930.60	28	1.13
	2017	29.28	24.66	95.56	81.90	764.00	29	0.81
July	2016	29.43	23.45	95.61	86.51	830.20	26	0.86
	2017	29.87	24.21	95.32	78.00	755.80	27	1.53
August	2016	29.82	24.47	95.48	78.93	438.40	23	2.10
	2017	29.89	24.23	95.75	80.20	837.60	26	1.28
September	2016	30.46	23.55	93.63	71.33	280.40	18	1.89
	2017	31.33	24.26	94.67	76.84	650.40	21	2.26
October	2016	32.37	23.24	92.51	63.93	64.40	5	2.78
	2017	32.16	24.13	95.29	69.49	309.60	16	4.05
November	2016	33.80	22.88	91.66	51.60	42.00	3	2.79
	2017	34.60	23.75	93.07	59.50	21.00	4	4.91
December	2016	34.00	21.12	91.90	44.41	20.00	2	2.58
	2017	35.00	22.00	89.39	49.97	15.20	2	5.22
January	2017	34.60	20.09	89.38	32.51	53.00	2	4.08
	2018	34.74	19.56	88.80	46.16	Nil	Nil	6.20
February	2017	35.50	21.80	88.17	35.00	53.00	1	4.90
	2018	36.34	20.57	88.28	47.50	Nil	Nil	7.00

APPENDIX II

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
MSS	Mean Sum of Squares
et al.	And others/co-workers
etc.	And so on
Df	Degrees of freedom
Cm	Centimeter
M	Meter
%	Per cent
Viz.,	Namely
°C	Degree Celsius
*	Significant
Min	Minutes
h	Hour
kg	Kilogram
g	Gram
l	Litre
mg	Milligram
ng	Nanogram
µl	Micro litre
ml	Milli litre
mM	Milli molar
NS	Non significant
Fig.	Figure
i.e.,	That is
Acc.	Accession
GCV	Genotypic Coefficient of Variation
PCV	Phenotypic Coefficient of Variation
GAM	Genetic Advance as Per cent Mean
RAPD	Random Amplified Polymorphic DNA
SSR	Simple Sequence Repeat
SNP	Single Nucleotide Polymorphism
PCR	Polymerase chain reaction
bp	Base pair
β	Beta
α	Alpha
CV	Coefficient of variation
CD	Critical difference