

**STUDIES ON UTILIZATION OF WILD SPECIES OF
GUAVA (*Psidium guajava* L.) FOR CROP
IMPROVEMENT**

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BAGALKOT – 587 104**

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**STUDIES ON UTILIZATION OF WILD SPECIES OF
GUAVA (*Psidium guajava* L.) FOR CROP
IMPROVEMENT**

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in

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By

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2017

*Affectionately Dedicated to my
Vapichi, Ummichi and Kunjikka*

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CERTIFICATE

*This is to certify that the thesis entitled “STUDIES ON UTILIZATION OF WILD SPECIES OF GUAVA (*Psidium guajava* L.) FOR CROP IMPROVEMENT” submitted in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (Horticulture)** in **FRUIT SCIENCE** to the University of Horticultural Sciences, Bagalkot, is a record of bonafide research work carried out by **Miss ALFIA, M. A.**, during the period of her study in this University, under my guidance and supervision and that no part of the thesis has been submitted for the award of any other degree, diploma, associateship, fellowship or other similar titles.*

Place: Bengaluru

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STUDIES ON UTILIZATION OF WILD SPECIES OF GUAVA (*Psidium guajava* L.) FOR CROP IMPROVEMENT

ALFIA M A

ABSTRACT

“Studies on utilization of wild species of guava (*Psidium guajava* L.) for crop improvement” was carried out at the Division of Fruit crops, ICAR-Indian Institute of Horticultural Research, Bengaluru during 2016-2017. The phenology of wild species, its crossability and the volatile aroma profile was carried out. The results revealed that synchronization was noticed for peak flowering (28 days), duration of flowering (60 days) and anther dehiscence (7.00-8.00 a.m) of *P. guineense* and *P. chinensis* (22, 58 and 8.00-10.00 a.m, respectively) with Arka Kiran (30, 65 and 7.00-9.00 a.m, respectively). On the basis of hybridization, *P. chinensis* was found to be a good combiner with cultivated varieties, among which, Arka Rashmi revealed high values (fruit set-97.07%). Cytological studies on cultivars and wild species revealed that chromosomes obtained were not in active mitotic metaphase. The fruit morphological studies revealed that fruits of wild species were small (*P. cattleianum*-22.90, *P. guineense*-31.41 and *P. chinensis*-29.70 g) with hard seeds (*P. guineense*-13.33 and *P. cattleianum*-11.17 kg cm⁻²) except *P. chinensis* (5.00 kg cm⁻²). The TSS, titrable acidity, vitamin C, total sugars, reducing sugar and non reducing sugar of wild species (*P. cattleianum*- 7.73⁰B, 1.28%, 242.53 mg 100⁻¹ g, 5.49 g 100⁻¹g, 4.45 g 100⁻¹g, 1.48 g 100⁻¹g, respectively, *P. guineense*-11.13, 0.60, 77.27, 7.67, 5.26, 3.52 respectively and *P. chinensis*-9.67, 0.69, 232.47, 8.56, 5.13, 3.13 respectively) was comparable whereas, total carotenoid and lycopene (*P. cattleianum*-7.60 and 2.30 mg 100⁻¹ g, respectively and *P. chinensis*-5.30 and 3.00 mg 100⁻¹ g) content was found to be inferior to the cultivars. The volatile aroma profile revealed that *P. chinensis* was rich in monoterpenoid (limonene-19.81%), *P. guineense* in sesquiterpenoid (α -Cedrene-41.10%) and alcohol (β -Bisabolol-3.12%), *P. cattleianum* in esters ((Z)- Valerenyl acetate-3.37%). Hence, the wild species could be exploited for transfer of these traits to the hybrids.

Alfia M. A.

15-11-17

(C. VASUGI)

Major advisor



ಸೀಬೆ ಬೆಳೆ ಸುಧಾರಣೆಗಾಗಿ ಕಾಡು ಪ್ರಭೇದದ ಬಳಕೆ ಅಧ್ಯಯನ (ಪ್ಲಿಡೀಯಂ ಗುಜಾವ ಎಲ್.)

ಅಲ್ಪೀಯ ಎಮ್. ಎ

ಅಮೂರ್ತ

ಸೀಬೆ ಬೆಳೆ ಸುಧಾರಣೆಗಾಗಿ ಕಾಡು ಸೀಬೆ ಪ್ರಭೇದಗಳ ಬಳಕೆ ಹಾಗೂ ಸಂಕರಣ ತಳಿ ಉತ್ಪಾದನೆಯ ಬಗ್ಗೆ ಅಧ್ಯಯನ ಮತ್ತು ಬಾಷ್ಪಶೀಲ ಸುವಾಸನೆಯ ಗುಣಲಕ್ಷಣಗಳ ಬಗ್ಗೆ ಅಧ್ಯಯನವನ್ನು 2016-17ನೇ ಸಾಲಿನಲ್ಲಿ ಭಾರತೀಯ ತೋಟಗಾರಿಕಾ ಸಂಶೋಧನಾ ಸಂಸ್ಥೆಯಲ್ಲಿ ಅಧ್ಯಯನ ಮಾಡಲಾಯಿತು. ಫಲಿತಾಂಶಗಳ ಪ್ರಕಾರ ಕಾಡು ಪ್ರಭೇದಗಳು ಮತ್ತು ಅರ್ಕಾ ಕಿರಣ್ ಹೂವು ಬಿಡುವುದು (ಪ್ಲಿಡೀಯಂ ಗೈನೇನ್ಸಿಸ್-28 ದಿನಗಳು, ಪಿ. ಚೈನೇನ್ಸಿಸ್-22 ಮತ್ತು ಅರ್ಕಾ ಕಿರಣ್-30), ಹೂವು ಬಿಡುವ ಸಮಯ (ಪಿ. ಗೈನೇನ್ಸಿಸ್-60 ದಿನಗಳು, ಪಿ. ಚೈನೇನ್ಸಿಸ್-58 ಮತ್ತು ಅರ್ಕಾ ಕಿರಣ್-65) ಮತ್ತು ಪರಾಗಶೋಶ ವಿಘಟನೆ ಕ್ರಿಯೆಗಳು (ಪಿ. ಗೈನೇನ್ಸಿಸ್- ಬೆಳೆಗೆ 7 ರಿಂದ 8 ಗಂಟೆ, ಪಿ. ಚೈನೇನ್ಸಿಸ್-8 ರಿಂದ 10 ಮತ್ತು ಅರ್ಕಾ ಕಿರಣ್-7 ರಿಂದ 9) ಏಕಕಾಲದಲ್ಲಿ ಉಂಟಾಗುವುದು ಕಂಡುಬಂದಿದೆ. ಮಿಶ್ರ ತಳಿ ಆಧಾರದ ಮೇಲೆ ಪ್ಲಿಡೀಯಂ ಚೈನೇನ್ಸಿಸ್ ಕಾಡು ಪ್ರಭೇದವು ಇತರ ತಳಿಗಳೊಂದಿಗೆ ಉತ್ತಮ ಸಂಯೋಜಕ ಎಂದು ಗುರುತಿಸಲಾಗಿದೆ. ಕಾಯಿ ಕಚ್ಚುವಿಕೆಯಲ್ಲಿ (ಬೆಳವಣಿಗೆ) ಅರ್ಕಾ ರಶ್ಮಿ ತಳಿಯು ಉತ್ತಮ ಎಂದು ಗುರುತಿಸಲಾಗಿದೆ (ಕಾಯಿ ಕಚ್ಚುವಿಕೆ 97.07%). ತಳಿಗಳು ಮತ್ತು ಕಾಡು ಪ್ರಭೇದಗಳ ಮೇಲಿನ ಜೀವತಂತುಗಳ ಅಧ್ಯಯನದಿಂದ ವರ್ಣತಂತುಗಳು ಸಕ್ರಿಯವಾಗಿ ಮೈಟಾಟಿಕ್ ಮೆಟಾಫೀಸ್ನಲ್ಲಿ ಲಭ್ಯವಾಗಿರಲಿಲ್ಲ. ಹಣ್ಣಿನ ಸ್ವರೂಪ ವಿಜ್ಞಾನದ ಅಧ್ಯಯನದ ಪ್ರಕಾರ ಕಾಡು ಪ್ರಭೇದದ ಹಣ್ಣುಗಳು ಚಿಕ್ಕದಾಗಿದ್ದು (ಪಿ. ಕ್ಯಾಟ್ಲಿಯಾನಂ -22.90, ಪಿ. ಗೈನೇನ್ಸಿಸ್-31.41 ಮತ್ತು ಪಿ. ಚೈನೇನ್ಸಿಸ್-29.70 ಗ್ರಾಂ), ಬೀಜವು ಗಟ್ಟಿಯಾಗಿತ್ತು (ಪಿ. ಗೈನೇನ್ಸಿಸ್-13.33 ಮತ್ತು ಪಿ. ಕ್ಯಾಟ್ಲಿಯಾನಂ-11.17 ಕಿಲೋ ಗ್ರಾಂ /ಚ.ಸಂ.ಮೀ). ಉಳಿದೆಲ್ಲಾ ಕಾಡು ಪ್ರಭೇದದ ಹಣ್ಣಿನ ತೂಕವು ಕಡಿಮೆಯಾಗಿತ್ತು (ಪಿ. ಚೈನೇನ್ಸಿಸ್-5.00 ಕಿಲೋಗ್ರಾಂ /ಚ.ಸಂ.ಮೀ). ಜೀವ ರಾಸಾಯನಿಕ ಗುಣಗಳಾದ ಒಟ್ಟು ಕರಗುವ ಸಕ್ಕರೆಗಳು, ಟೈಟ್ರಟಿಬಲ್ ಆಮ್ಲತೆ, ವಿಟಮಿನ್ ಸಿ, ಒಟ್ಟು ಸಕ್ಕರೆ, ಸ್ರವಿಸುವ ಸಕ್ಕರೆ, ಸ್ರವಿಸದ ಸಕ್ಕರೆಗಳನ್ನು ಕಾಡು ಪ್ರಭೇದ ಮತ್ತು ತಳಿಗಳಲ್ಲಿ ಹೋಲಿಸಬಹುದಾಗಿದೆ (ಪಿ. ಕ್ಯಾಟ್ಲಿಯಾನಂ -7.73⁰ಬ್ರಿಕ್ಸ್, 1.28%, 242.53ಮಿಲಿ ಗ್ರಾಂ/100ಗ್ರಾಂ, 5.49 ಗ್ರಾಂ/100ಗ್ರಾಂ, 4.45 ಗ್ರಾಂ/100 ಗ್ರಾಂ, 1.48 ಗ್ರಾಂ/100ಗ್ರಾಂ ಅನುಕ್ರಮವಾಗಿ, ಪಿ. ಗೈನೇನ್ಸಿಸ್-11.13, 0.60, 77.27, 7.67, 5.26, 3.52 ಅನುಕ್ರಮವಾಗಿ ಮತ್ತು ಪಿ. ಚೈನೇನ್ಸಿಸ್ 9.67, 0.69, 232.47, 8.56, 5.13, 3.13 ಅನುಕ್ರಮವಾಗಿ). ಆದರೆ ಒಟ್ಟು ಕೆರೋಟಿನಾಯ್ಡ್‌ಗಳು ಮತ್ತು ಲೈಕೋಪಿನ್ ಅಂಶಗಳು ತಳಿಗಳಿಗೆ ಹೋಲಿಸಿದರೆ ಕಾಡು ಪ್ರಭೇದಗಳಲ್ಲಿ ಕಡಿಮೆಯಾಗಿರುತ್ತದೆ (ಪಿ. ಕ್ಯಾಟ್ಲಿಯಾನಂ -7.60 ಮತ್ತು 2.30 ಮಿಲಿ ಗ್ರಾಂ/100 ಗ್ರಾಂ ಅನುಕ್ರಮವಾಗಿ ಮತ್ತು ಪಿ. ಚೈನೇನ್ಸಿಸ್-5.30 ಮತ್ತು 3.00 ಮಿಲಿ ಗ್ರಾಂ/100ಗ್ರಾಂ). ಬಾಷ್ಪಶೀಲ ಪರಿಮಳದ ಅಧ್ಯಯನದಿಂದ ಕಾಡು ಪ್ರಭೇದಗಳಾದ ಪಿ. ಚೈನೇನ್ಸಿಸ್‌ನಲ್ಲಿ ಹೆಚ್ಚು ಮೊನೋಟರ್‌ಪೆನ್ಯಾಯ್ಡ್ (ಲಿಮೋನನ್-19.81%) ಪಿ. ಗೈನೇನ್ಸಿಸ್‌ನಲ್ಲಿ ಹೆಚ್ಚು ಸೆಸ್ಕ್ವಿಟರ್ಪೆನಾಯ್ಡ್ (ಅಲ್ಫಾ ಸಿಟ್ರಿನ್, 41.10%) ಮತ್ತು ಅಲೋಹಾಲ್ (ಬೀಟಾ ಬಿಸಾಬೋಲೋಲ್ 3.12%) ಪಿ. ಕ್ಯಾಟ್ಲಿಯಾನಂ‌ನಲ್ಲಿ ಹೆಚ್ಚು ಈಸ್ಟರ್ಸ್ (ಚೆ. ವೆಲಿರೆನಲ್ ಅಸಿಟೇಟ್ 3.37%). ಆದ್ದರಿಂದ ಈ ಕಾಡು ಪ್ರಭೇದಗಳ ಈ ಮೇಲಿನ ಕೆಲ ಒಳ್ಳೆಯ ಅಂಶಗಳನ್ನು ತಳಿಗಳಿಗೆ ವರ್ಗಾವಣೆ ಮಾಡಲು ಬಳಸಿಕೊಳ್ಳಬಹುದಾಗಿದೆ.

ಅಲ್ಪೀಯ ಎಮ್. ಎ.

15.11.2017

(ಸಿ. ವಾಸುಕಿ)

ಪ್ರಮುಖ ಸಲಹೆಗಾರರು

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ABBREVIATIONS AND SYMBOLS USED

%	- Per cent
$^{\circ}\text{C}$	- Degree Celsius
NHB	- National Horticulture Board
Viz.,	- Namely
min	- Minutes
m	- Meter
cm	- Centimeter
g	- Gram
mg g^{-1}	- Milligram per gram
$\text{SEm}\pm$	- Standard error of mean
CRD	- Completely randomized block design
RCBD	- Randomized complete block design
MgSO_4	- Magnesium sulphate
CaNO_3	- Calcium nitrate
KNO_3	- Potassium nitrate
ppm	- Parts per million
M	- Molar
N	- Normal
ml	- milliliters
nm	- nanometer
Kg cm^{-2}	- Kilogram per centimeter square
$^{\circ}\text{B}$	- Degree Brix
CaCO_3	- Calcium chloride

TSS	- Total soluble solids
NaCl	- Sodium chloride
Na ₂ SO ₄	- Sodium sulphate
NaOH	- Sodium hydroxide
FW	- Fresh weight
CD	- Critical difference
EDTA	- Ethylene diaminetetraacetic acid
HCl	- Hydrochloric acid

I. INTRODUCTION

Guava (*Psidium guajava* L., $2n = 22$) belongs to the family Myrtaceae, is one of the most important commercial fruit crops of India. It is considered as the 'apple of the tropics' and 'poor man's apple' for its high vitamin C and mineral contents. The origin of guava, although uncertain, is believed to be Central and South America (Nakasone and Paul, 1998). The genus *Psidium* includes about 150 species among which only twenty produce edible fruits and is widely distributed all over the equatorial regions of tropical and subtropical climates. Commercial cultivation of guava is common in India, Brazil, Mexico, Florida, Hawaii, California etc.

Due to its ability to grow in tropical and subtropical climates, the guava tree has been introduced into many countries. In the Indian subcontinent, it is reported to have been in cultivation since early 17th century (Mitra and Bose, 1983). Because of its hardiest nature, it has very well adapted to a wide variety of soil and climatic condition of India and it gives assured yield with minimum care.

Guava fruit shows a quite interesting nutritional profile. It is a very rich and cheap source of vitamin C (100-260 mg 100 g⁻¹ of pulp) and contains a fair amount of calcium also. Fruits are used for jelly preparation because of rich pectin content. It also contains vitamin A, phosphorus and small quantities of thiamine, riboflavin and niacin (Radha and Mathew, 2007).

It is the fifth most widely grown fruit crop of India. The area under guava is about 2.62 million hectares with a production of 3.65 MT of fruits (NHB, 2016-17). The popular varieties of guava are Allahabad Safeda, Sardar (Lucknow-49), Chittidar, Apple Colour, Arka Mridula, Lalit, Shwetha, Arka Kiran, Hafsi, Benaras, Smooth Green, Hisar Safed, Hisar Surkha, etc. Uttar Pradesh is the leading state in guava production with 0.937 MT followed by Madhya Pradesh, Bihar, Maharashtra, West Bengal, Punjab, Chattisgarh, Haryana (NHB, 2014-15).

Adequate genetic variation has been observed in *P. guajava* for the development of commercial cultivars with economic importance. *P. guajava* is the most commonly cultivated and widely studied species (Pathak and Ojha, 1993). Other *Psidium* species including *P. cattleianum* Sabine, *P. friedrichsthalianum* (O. Berg) Nied., *P. guineense* Sw., *P. montanum* Sw. and *P. sartorianum* (O. Berg) Nied are fairly well distributed and used for crop improvement.

The economic importance of guava has driven significant efforts to accelerate the exploitation of *Psidium* genetic resources for development of new cultivars towards biotic and abiotic stress improvement. However, in perennial crops because of the long time gap, efforts have not been made to their full potential. Although success obtained in fruit crops is low, many desirable traits with great potential for crop improvement are found in the wild species. But if the species are incompatible, then these traits cannot be exploited properly. So, it is important to study the compatibility between different wild species with *P. guajava* cultivars. As stated by Usman *et al.*, 2013, recombination breeding between varieties and wild species is one of the major approaches of guava improvement.

The growing cycle depends on plant genotypes as well as on climatic conditions. Identical genotypes when grown under different conditions may show different developmental stages. In order to make use of different genotypes for effective crop improvement programme, it is crucial to know their phenological stages. As these studies help to bring out precise information about the flowering behavior, duration of flowering, anthesis and pollen dehiscence, time taken for fruit set, duration of fruiting etc among the cultivated and wild species, it helps to plan an effective hybridization programme involving the wild species and cultivated varieties. The interval between blooming and fruit set is one of the most important phenological stages (Villalpando and Ruiz, 1993).

The chromosome study is important for crop improvement by forecasting compatibility. Aroma profiling helps in utilizing aroma as a marker to observe the transfer of gene, as transfer of any trait may lead to change in aroma compound of the hybrid.

Hence, the present study deals with the phenology of wild species and its crossability with *P. guajava* cultivars, cytological studies and volatile aroma profiling of cultivars and wild species of guava that aims at crop improvement. Keeping this information as baseline, an investigation entitled 'Studies on the utilization of wild species of guava (*Psidium guajava* L.) for crop improvement' has been carried out with following objectives.

Objectives of investigation

1. To study the phenology of wild species of guava viz., *P. guineense*, *P. chinensis* and its crossability with *P. guajava* L. cultivars viz Arka Kiran, Arka Rashmi and Arka Mridula.
2. To study the cytology of *P. guajava* varieties and wild species of guava.
3. To study the morphological, biochemical traits and volatile aroma profile of fruits of *P. guajava* cultivars and wild species of guava.

II. REVIEW OF LITERATURE

Guava (*P. guajava* L., $2n = 22$) belongs to the family Myrtaceae, is one of the most important commercial fruit crops of India. It is considered as the 'apple of the tropics' and 'poor man's apple' for its high vitamin C and mineral contents. The origin of guava, although uncertain, is believed to be Central and South America (Nakasone and Paul, 1998). The genus *Psidium* includes about 150 species among which only twenty produce edible fruits and is widely distributed all over the equatorial regions of tropical and subtropical climates.

Ripe guava is a very rich and cheap source of vitamin C (100-260 mg 100 g^{-1} of pulp) and contains a fair amount of calcium also. Fruits are used for jelly preparation because of rich pectin content. Fruits contain phosphorus, iron and small quantities of thiamine, riboflavin and niacin (Radha and Mathew, 2007).

Adequate genetic variation has been observed in *P. guajava* for the development of commercial cultivars with economic importance. *P. guajava* is the most commonly cultivated and widely studied species (Pathak and Ojha, 1993). Other *Psidium* species including *P. cattleianum* Sabine, *P. friedrichsthalianum* (O. Berg) Nied., *P. guineense* Sw., *P. montanum* Sw. and *P. sartorianum* (O. Berg) Nied are fairly well distributed and used for crop improvement. Efforts have been made over the past few decades to widen guava genetic base. Attempts are still going on to improve the existing commercial cultivars through selection of germplasm and combining the desirable traits of various genotypes through hybridization. A variety with in-built resistance to the biotic and a biotic resistance besides high yielding capacity of good quality fruit is lacking in guava.

Utilization of wild species for crop improvement has been one of the ways to introduce certain gene(s) for imparting resistance to biotic and abiotic stresses through hybridization and also as a rootstock for imparting specific traits. Exploitation of wild species requires extensive knowledge of taxonomy, reproductive biology & cytogenetics, genetics, crossability barriers and fertility of the hybrids. The works carried out on these aspects in guava as well as other fruit crops are reviewed as follows:

2.1 Phenology of wild species of guava

The study of the sequence of all periodical events involved in a plant life cycle is called 'phenology'. Plant growth rates can be evaluated by establishing the beginning and end of every developmental stage (Torres, 1995). The interval between two different phases is called 'phenological stage' (Villalpando and Ruiz, 1993).

A brief description of some of the wild species of guava which are having potential in use in guava improvement are as follows:

***Psidium cattleianum* Afzel. ex Sabine (syn: *P. cattleianum* var *longipus* (O.Berg) Fosberg, *P. cattleianum* var. *littorale* Raddi, *P. littorale* Raddi, *P. cattleianum* var *lucidum*.Sabine**

It is commonly called as cattley guava, cherry guava, purple guava, red strawberry guava, strawberry guava, yellow strawberry guava etc. It is native of South East Brazil and North Uruguay, but now naturalized throughout the tropics and subtropics. It is also known as 'strawberry guava' because of the sweet aroma reminiscent of strawberry. Fruits of red strawberry guava are red and that of yellow strawberry guava are light yellow to sulphur yellow colour skinned. Fruits are berries with round shape and about 2 to 4 cm diameter. The white soft and juicy flesh contains several hard seeds. It has sweet flavor and good aroma. It lacks muskiness of common guava. It is cold hardy (Julian, 1998) species. It is also reported to be resistant to wilt (Landrum *et al.*, 1995 and Edward and Shankar, 1964). Singh *et al.* (1977) reported that high yields were obtained while using *P. cattleianum* as rootstock.

***Psidium guineense* Sw.** (Syn: *P. araca* Raddi, *P. molle* Bertol, *P. monticola* O. Berg, *P. schedianum* O. Berg).

It is commonly called as Brazilian guava or Castilian guava or Guinea guava. The flavour is acidic. It is reported to have drought hardy nature. Singh *et al.* (1977) reported that it is resistant to wilt.

***Psidium chinensis*:**

The plants are medium tall with narrowly elliptic shaped leaves. The fruits are globose with light yellow coloured rind when it reaches maturity, sweet in taste. Fruit consist of small, medium soft seeds. The fruit is having an average TSS of 8.3⁰ B, and high vitamin C content (231.5mg 100g⁻¹pulp). The average titrable acidity is 0.6 % and total sugar of 9.34 g 100g⁻¹ pulp (Vasugi and Dinesh, 2007).

***Psidium friedrichsthalianum* Niedenzu** (Syn: *Calyptropsidium friedrichsthalianum* O. Berg.):

It is called as 'Chinese guava' or 'Costa Rican guava'. Fruits are good for jelly making because of its high acidity (Villota and Agudelo, 1997). It is reported to have resistance to guava wilt (Landrum *et al.*, 1995)

***Psidium acutangulum* Mart. Ex DC** (Syn: *Guajava acutangula* (Mart. Ex DC) Kuntze, *Psidium acidum* Mart. Ex O. Berg, *Psidium grandiflorum* Ruiz & Pav. nom. illeg.):

It is commonly known as para guava. The fruits contain a few hard-triangular seeds. It is moderately resistant to wood eating organisms (Lorenzi, 2009).

***Psidium montanum* (Swartz)**

It is found generally in mountains of Jamaica. The branchlets are four angled, leaves oblong to oval, glabrous, fruits are round, pulp white with more number of seeds. It produces fruits of poor quality

***Psidium araucanum* (Soares-Silva and Proenca):**

A large tree with membranous leaves, brochidodromous venation, long petioles and peduncles, Flowers solitary, axillary or ramiflorous, or in short racemes with two pairs of flowers. Fruits globose or pear shaped, thin pericarp, fruits yellowish green when mature, seeds angular or lenticulate

Psidium angulatum

Small tree, leaves are alternate and oblong. Fruit has a leathery peel and persistent calyx (Castellane, 2005). Fruits are eaten raw or used to flavour drink, processed into ice cream, sorbet, gelatins and candies. Peels contain compounds with high antioxidant activity. Vitamin C 389.3 mg 100 g⁻¹ (Aecoturis, 2005).

Sixteen different phenological stages were identified in guava during the annual growing cycle, starting at bud dormancy and ending at fruit ripening. This included winter bud, bud swelling, beginning of bud growth, first leaves sprouting, more leaves unfolding, complete development of leaves, appearance of flower buds, flower bud visibility, flower petals elongation, sepals totally opening, full flowering, petal fall, fruit setting, fruit growth, fruit colour changing and fruit ripening (Salazar *et al.*, 2006).

As a result of seasonal changes, most fruit tree species showed clearly defined phenological stages both during winter dormancy and the active vegetative growth period (Champagnat, 1965). The period of vegetative growth begins at mid spring and finishes in autumn. During this period the guava tree (*P. guajava* L.) undergoes several physiological changes that are identified because of external signs

such as shoot growth, bud swelling and break, increments in trunk diameter as well as reproductive phases like flower initiation, fruit setting and ripening (Salazar *et al.*, 2006)

Several phenological indicators are used to monitor and evaluate plant development. The most significant ones for fruit tree species are blooming time and fruit ripening. The interval between blooming and fruit set is one of the most important phenological stages (Villalpando and Ruiz, 1993).

Laguado *et al.* (1999) and Mercado-Silva *et al.* (1998) found that the stage from fruit set to guava physiological maturity lasted 119 and 130 days, respectively; Somarriba (1985) also indicated that guava reaches maturity 90–150 days after anthesis.

As a basis for hybridisation and improvement of cultivated *P. guajava*, Seth (1963) established comparative studies of the floral morphology, floral biology, incompatibility, cytology, embryology and seed development of *P. guajava*, *P. guineense*, *P. chinense*, *P. molle* and *P. cattleianum* var. *lucidum*. Floral biology of all the species was reported to be very similar to one another. *P. cattleianum* differed slightly in several ways from the other species.

2.2 Effect of pruning on flowering and fruiting

In a study conducted in Brazil to evaluate the phenological characteristics, yield and fruit quality of the 'Paluma' guava tree at different pruning times, the plants pruned on August took 46, 72 and 84 days after pruning (DAP) for the branch establishment phase, flowering (anthesis) and early fruiting characteristics respectively. From early fruiting to early maturation stage it took an average of 120 days. It took around 204 DAP to reach fruit maturation in August pruned plants. Guava trees pruned on August, September and October took around 211, 192, 195 days after pruning from fruit pruning to harvesting respectively. The longest time interval among the assessed phenological phases occurred between the early fruiting and early fruit maturation stages, which was expected because this is the fruit growth period for both length and diameter (da Silva *et al.*, 2016).

In a study conducted in Mexico with an aim to study the phenological development of guava trees and their relationships with ambient temperature at three locations of Mexico having different climatic conditions, a location with annual mean temperature fluctuates from 25 to 18°C showed the duration from pruning to flowering (P-F) ranges from 60 to 115 days. Guava trees require about 800 to 850 heat units for stages from pruning to flowering (P-F) (Padilla-Ramirez *et al.*, 2012).

2.3 Flowering

A study conducted by Padilla-Ramirez *et al.* (2012) with an aim to study the phenological development of guava trees and their relationships with ambient temperature at three locations of Mexico having different climatic conditions reported that at a location with annual mean temperature fluctuates from 25 to 18°C, duration from flowering to beginning of harvest (F-BH) varies from 100 to 180 days and the trees require about 1,950 to 2,000 heat units for flowering to beginning of harvest (F-BH).

A study conducted on strawberry guava (*Psidium cattleianum* Sabine) at 100, 480 and 720m over 2 years in Reunion Island, France revealed that the flowering is related to shoot production and they bore flower buds at the basal nodes and vegetative buds at the apical nodes. Trees produced vegetative growth from September to December, flowering from November to January. Bud burst, shoot and flower bud development are completely temperature dependent which causes a difference in flowering period according to elevation (Normand and Habib, 2001).

In a study conducted by Normand *et al.* (2002) in Reunion island, France, bud bursting in strawberry guava (*P. cattleianum*) occurred during October independent of elevation. Later shoot and flower bud differentiation occurred followed by fruit growth.

In a study conducted on varietal evaluation and floral biology studies in the genus *Psidium*, it was reported that for the complete development of flower bud from 2 mm to full bloom during winter, it takes 45–51 days. Flower development in *P. quadrangularis*, *P. molle*, *P. cattleianum* and *P. friedrichsthalianum* take 36–45 days. Flowering in all the cultivars of *P. guajava* occurs three times a year as in *P. quadrangularis*. In *P. friedrichsthalianum* few flowers are observed between two flushes (Subramanyam *et al.* 1992).

According to Ray (2002) in India, the duration of flowering in each of the two peak flowering seasons is 35 to 45 days. The fruit bud differentiation is practically continuous throughout the year except during winter.

In a study conducted by Seth (1963) on floral morphology, floral biology, incompatibility, cytology, embryology and seed development of *P. guajava*, *P. guineense*, *P. chinense*, *P. molle* and *P. cattleianum* var. *lucidum*, it was reported that maturation of floral buds was most rapid in *P. cattleianum*.

Flower development takes about 30 days from flower bud differentiation to calyx cracking stage (Subramanyam and Iyer, 1993) and the cracking of the calyx occurs nearly 24 hours before flower opening. However, in a study conducted by Sehgal and Singh (1967) on blossom biology of guava, it has been reported that calyx splitting can happen anywhere between 13 and 26 hours before flower opening.

When the flower buttons reach their maximum development, the sepals begin breaking up in several points, signalling the beginning of anthesis. The following day, at around 6 a.m., for approximately an hour, the gradual opening of almost all the buttons begins (Soubihesobrinho, 1951) and is variable at the beginning and depends on diurnal temperature.

2.4 Pollen germination

In *P. cattleianum* anther dehiscence was found to be after anthesis rather than before anthesis as in the other species; pollen had the lowest viability; optimum temperature for pollen germination was lower (25°C as opposed to 30°C for the other species); pollen longevity was shortest, the stigma becoming receptive the day the flower opens, remaining so for up to 72 hrs. (in other species, the stigma became receptive the day after anthesis and remained so up to 32 hrs.) (Seth, 1963). Hirano (1967) also observed that pollen of *P. cattleianum* var. *lucidum* could not be germinated.

In a study conducted by Hirano and Nakasone (1969) on pollen germination and crossing studies in *P. cattleianum*, *P. cattleianum* f. *lucidum*, *P. guajava*, *P. guineense*, *P. cujavillus* and *P. friedrichsthalianum*. Pollen of *P. guajava* (cultivars used had $n = 22$ and $n = 33$) generally had high germination rates, which were higher than those for species with higher chromosome numbers. Pollen of *P. cattleianum* showed 32% germination, while that of *P. cattleianum* f. *lucidum* failed to germinate. Pollen tubes of *P. cattleianum* were shorter than those of other species.

Dehiscence of anthers and receptivity of the stigma occur just after the opening of the flower. As stated by Souza (1998), pollen viability was 99.59% and frequency of diads and triads was 0.25%. The flower buds that open the next morning show a cracking of the calyx on the previous day, nearly 24 hours in advance.

In guava, anthesis starts at 4.00 a.m. and continues till 10.00 a.m., with peak opening occurs between 5.00 and 7.00 a.m. Anther dehiscence starts 15–20 minutes after flower opening. Peak dehiscence time in majority of the cultivars was found to be 6.00–8.00 a.m. In *P. friedrichsthalianum*, peak dehiscence occurs between 7.00 and 9.00 a.m., while in *P. cujavillus* and *P. cattleianum* it is between 9.00 and 11.00 a.m. Viability of the freshly collected pollens varies from 42 to 95% depending upon the varieties. Seedless cultivars, in general, have less than 50% pollen viability whereas the seeded

varieties like Chittidar or Allahabad Safeda show over 90% pollen viability at the time of dehiscence (Ray, 2002).

2.5 Duration of fruiting

In a study conducted by Subramanyam *et al.* (1992), it was reported that, fruit set varies between 40 and 85.7% in *P. cattleianum*. Among the varieties of *P. guajava*, it ranges between 30.4% (cv. Pear Shaped) and 80% (cv. Allahabad Safeda and Apple Colour).

In guava, from fruit set to maturity, fruit was showing a single sigmoid curve in which stage I, stage II and stage III were 56, 56-84, 12 days respectively (Garce's, 1987, Barcelo' *et al.*, 1992, and Coombe, 1976).

In a study conducted at Mexico on fruit development, harvest index and ripening changes of guavas produced in central Mexico by Mercado-Silva *et al.* (1998) the results showed that, the fruit development of guava cultivar, 'Media China' depends on the season in which it is produced. The development of fruit followed a double sigmoidal pattern. The spring–summer crop took lesser days (130 days) to develop while autumn–winter crop required 190 days to reach the ripe stage.

A study conducted on strawberry guava (*P. cattleianum* Sabine) revealed that the number of fruits produced per branch depends on poor shoot growth or fruit set and is not related to the timing of the phenological events. Bud burst, shoot and flower bud development and fruit growth are completely temperature dependent which causes a difference in fruiting periods according to elevation (Normand and Habib, 2001). Generally, fruiting was from February to June.

In a study conducted by Normand *et al.* (2002) in Reunion island, at 500 m altitude, fruit of strawberry guava (*P. cattleianum*) matured by the end of rainy season (April-May) whereas at 1000-1200 m altitude it matured during cool and dry season (July-August). Fruiting lasted upto 1.5 to 2 months in a single location.

2.6 Interspecific hybridization

Improvement in guava fruit can be achieved through development of cultivars with least number and soft seeds, resistant to guava wilt disease and to fruit fly, whitefly and mealy bug, spreading growth habit, suitable for processing, better fruit quality in terms of shape, size, colour, firmness, thick pulp pleasing aroma and better keeping quality, having pink or red flesh and skin for export market (Shukla *et al.*, 2004).

One of the major approaches to address the issues (*viz.*, overcoming the severity of wilt diseases, high seed content of diploid varieties, poor yield with small, irregular and misshapen fruits of triploid seedless varieties) in guava cultivation is recombination breeding between varieties and wild species. Hybrid guava production significantly increased crop production thereby increases the economic returns of farmers and nation (Usman *et al.*, 2013).

According to Pereira and Nachtigal (2002), the first step of the work is the selection of the parents, in order to make possible the combination of favourable characters in the descendants to be selected.

Interspecific crosses in many fruit crops, between cultivars and species, have resulted in hybrids that are partially sterile due to ploidy level differences, genomic incompatibilities and cytoplasmic imbalances. In order to develop a rootstock tolerant/resistant to guava wilt and to test the possible role of different species, studies have been initiated on interspecific hybridization, in the genus *Psidium*. It was

observed that *P. guajava* and *P. chinensis* were crossable. However, *P. guajava* and *P. molle* are cross incompatible when *P. guajava* is used as the female parent (Subramanyam and Iyer, 1982).

Landrum *et al.* (1995) and Edward and Shankar (1964) reported that *P. freidrichsthalianum* Niedenzu to be resistant to guava wilt. The other species reported to be resistant to the wilt are *P. cumuni*, *P. cattleianum* var. *lucidum*, *P. molle*, and *P. guineense*.

P. cattleianum and *P. cattleianum* var. *lucidum* could be crossed in both directions; *P. guajava* could not be crossed with *P. cattleianum* or with *P. cattleianum* var. *lucidum*. *P. cattleianum* and *P. cattleianum* var. *lucidum* could not be crossed with *P. guineense*, *P. cujavillus* or *P. friedrichsthalianum* (Hirano, 1967).

Seth (1960) reported varietal cross incompatibility since neither fruit nor seed set was obtained when crosses were made between Behat Coconut × Lucknow-49, S1 × Behat Coconut, Behat Coconut × Apple Colour and Apple Colour × S1. Triploidy and some other genetic factors have been reported to be responsible for female sterility. The variation observed in triploids was possibly due to their independent origin from a different diploid variety. At Coimbatore, in the triploid fruits, a black mass of degenerated ovules was observed in the centre due to less stimulation by placenta during fertilisation. There is a need to further study qualitative and quantitative inheritance in order to assist the guava breeder in interpreting phenotypic values in terms of potential genetic gain.

2.7 Interspecific hybridization for nematode resistance

Molero *et al.* (2010) showed the possibility of obtaining pest-resistant cultivars with fruit of excellent quality. Authors tested resistance to nematodes *Meloidogyne enterolobii* and *M. incognita* and found selections of *P. guajava* tolerant and one of *P. friedrichsthalianum* as resistant.

Da Costa *et al.* (2012) assessed the interspecific hybrids (*Psidium guajava* acc. GUA 161 PE × *P. guineense* acc. ARA 138PE and ARA 153 BA) for its tolerance to nematode (*Meloidogyne enterolobii*). Five of the 33 plants of the ARA 138 RR accession were susceptible to *M. enterolobii*, while 2 of the 8 plants of the ARA 153 BA accession were susceptible and the others presented resistance to the nematode. These results indicated that there is among and within variability in *P. guineense* accessions for *M. enterolobii*, interspecific hybrids between GUA 161 PE × ARA 138 RR were highly tolerant to the nematode, interspecific hybrids between GUA 161 PE × ARA 138 RR have presented similar growth to that of the guava trees and high compatibility with 'Paluma'.

Maranhão *et al.* (2001) and Miranda (2011) also reported the existence of within variability in species tolerant to the nematode in wild *Psidium* species. Tolerance to *M. enterolobii* has been reported in *P. guineense* (Maranhão *et al.*, 2003), *P. friedrichsthalianum* and *P. cattleianum* (Carneiro *et al.*, 2007). Conflicting results indicating susceptibility have been reported for *P. guineense* (Miranda, 2011) and *P. friedrichsthalianum* (Almeida *et al.*, 2009).

The search for the source of tolerance to *M. enterolobii* in wild *Psidium* species (Cuadra and Quincosa, 1982; Carneiro *et al.*, 2007; Almeida *et al.*, 2009) resulted in identifying tolerance sources, but has presented limited or complete incompatibility when used as rootstock for commercial guava trees cultivars (Carneiro *et al.*, 2007; Almeida, 2008; Castro *et al.*, 2012). The interspecific hybrids between *Psidium guajava* × *P. guineense* (161 PE × ARA 138 RR) cross assessed were tolerant to the nematode, with gall index and RF equal to zero. Seven of the 10 plants of the GUA 161 PE × ARA 153 BA cross assessed were considered susceptible to the nematode. The results suggested variability for tolerance among *P. guineense* accessions and that tolerance to the nematode could be conferred by a dominant allele. The interspecific hybrid is highly compatible with 'Paluma' guava, indicating that this strategy can present excellent results in controlling *M. enterolobii*.

At the Central Institute for Subtropical Horticulture, Lucknow, Uttar Pradesh, interspecific hybridization was done between *P. molle* and *P. guajava*. The interspecific hybrids have been found resistant to guava wilt and are graft compatible with commercial varieties of *P. guajava* (Anon., 2003-04). The wilt resistant hybrid rootstock of guava (*P. molle* × *P. guajava* L.) is advanced for field evaluation by grafting on commercial scions in wilt endemic areas / multi-location testing through AICRP (STF) network and KVKs (Anon., 2014-15).

In the hybridisation programme, controlled crossings are affected using selected parents that are having distinct traits. Then field evaluation of the hybrid progeny population will be carried out. Hybridity will be tested based on the morphological traits of the parents. The traits viz., colour of young twig, anthocyanin colouration of young leaf, chlorophyll content of leaf and leaf morphological characters similar to both male and female parents along with unique flesh and skin colour may be useful in evaluating the progenies (Singh *et al.*, 2013).

Hybrids from interspecific crosses are easily detectable morphologically because of the distinct morphological characters of the parents. Existence of natural hybrids between *P. guajava* and *P. guineense* were reported in Latin American regions through morphological and biochemical traits (Landrum *et al.*, 1995).

Natural existence of triploids which generated seedlessness was reported in guava (Kumar and Ranade, 1952 and Raman *et al.*, 1971). Triploid and diploid crosses were derived in aneuploids viz., trisomics, double trisomics, tetrasomics and higher aneuploids. Morphological characterization of these hybrids was substantiating by the reduction of growth and size in lower aneuploids and exaggerated growth in higher aneuploids (Mohammad, 1974).

P. guineense, wild species of guava was known for its tolerance to the nematode, *Meloidogyne enterolobii*. An interspecific hybrid was made to introgress biotic tolerance to the cultivated species *P. guajava*. True hybrids were confirmed using microsatellite markers mPgCI 251 and mPgCI 252. Tests were also done to confirm the introgression of genes responsible for nematode tolerance through scoring gall or egg masses (Da Costa *et al.*, 2012).

Singh *et al.* (1977) reported none of the seven species, *P. arcae*, *P. cattleianum*, *P. cattleianum* var. *lucidum*, *P. corecium*, *P. cujavillus*, *P. guineense* and *P. fridichsthalium* developed wilt infection.

Since interspecies and intergeneric graft compatibility is possible, Edward (1961) suggested guava species *P. cattleianum* var. *lucidum* and *Syzigium cumini* (Jamun), which seldom get attacked with wilt, may be an effective way for the control of wilt disease. Edward and shanker (1964) in their further studies found *P. cattleianum* (*P. molle*), *P. quianense*, Chinese guava (*P. friedrichsthalium*) and Philippine guava are compatible and suggested them for the use of rootstock. A local variety Pei-pa In Taiwan was reported resistant and *P. friedrichsthalium* has been recommended as possible rootstock (Leu and Kao, 1979).

P. friedrichsthalium (Berg) Ned (Costa Rican wild guava) was considered resistant to *Meloidogyne enterolobii* (*M. mayaguensis*, *M. incognita* race 1 and race, *M. arenaria*, *M. javanica*) in different countries: Venezuela (Casassa *et al.*, 1998; Molero *et al.*, 2010), Colombia (Villota and Agudelo, 1997) and Brazil (Carneiro *et al.*, 2012).

According to Carneiro *et al.* (2012) Costa Rican wild guava *P. friedrichsthalium*, *P. cattleianum* (yellow wild guava), *Acca sellowiana* and *P. rufum* presented resistance or immunity to *M. enterolobii* at Brazil. One accessions of *P. friedrichsthalium* (Costa Rican wild guava) was resistant and compatible as rootstock with *P. guajava* 'Paluma', in field conditions.

Castro *et al.* (2012) have studied the reaction of *Psidium* accessions to the *Meloidogyne enterolobii* at Brazil. Among the 20 araçazeiro accessions evaluated in the greenhouse, three were

resistant (RF<1) and 9, were immune (RF=0). But Cuadra and Quincosa (1982) who screened 5 *Psidium* species in Cuba found that Costa Rican guava (*P. friedrichsthalianum*) was highly resistant to *Meloidogyne* spp.

Even though several studies have been conducted using wild species as either rootstock or in the hybridisation programme, intensive work is still needed to develop useful interspecific hybrids having resistance to wilt and nematode complex and further evaluation under different agro-ecological zones producing guava is urgently needed.

2.8 Breaking crossability barriers

In a study conducted on intergeneric hybridization in *Carica papaya* and *Vasconcellea cauliflora*, it was also observed that enhanced fruit was realized by the use of nutrient solution 5% (Sucrose 5% + Boric acid 100 ppm + MgSO₄ 200 ppm + CaNO₃ 300 ppm + KNO₃ 100 ppm) which is mainly due to the supply of required nutrients like boron, magnesium, calcium and potassium (Jayavalli *et al.*, 2011). In a similar study conducted in papaya by Dinesh, *et al.* (2007) on intergeneric hybridization of papaya, sucrose 5% was used to break the reproductive barrier.

2.9 Fruit set

Jayavalli *et al.* (2011) on intergeneric hybridization in *Carica papaya* and *Vasconcellea cauliflora*, reported that out of 1197 flowers pollinated using different nutrient combinations, initial fruit set was recorded to be 676 fruits and number of fruits reached harvest stage was 308 from which 721 intergeneric F₀ seeds were obtained from three varieties viz., CO 7, Pusa Nanha and CP 50. The cross combinations viz., CO 7 × *V. cauliflora*, Pusa Nanha × *V. cauliflora* and CP 50 × *V. cauliflora* produced 45, 67 and 400 numbers of seeds respectively with sucrose 5%. However, CP 50 × *V. cauliflora* crosses smeared with sucrose 5% + boron 0.5% had 200 seeds and sucrose 5% + CaCl₂ 0.5% produced 9 seeds.

Interspecific hybridization has been attempted to develop rootstocks resistant to guava wilt (Edward and Shankar, 1964). Work on interspecific hybridization involving Arka Mridula × *Psidium guineense* recorded 76.99% fruit set followed by Arka Kiran × *P. guineense* with a fruit set of 68.66 % at IIHR, Bengaluru (Das *et al.*, 2016).

2.10 Cytological studies in *P. guajava* cultivars and wild species of guava

The cytological markers are classical markers used in genetics and plant breeding not only for species identification but also to find out the structural features of chromosomes that can be shown by chromosome karyotype and bands. The banding patterns, displayed in colour, width, order and position, reveal the difference in distributions of euchromatin and heterochromatin. It helps in characterization of normal chromosomes and detection of chromosome mutation, but also widely used in physical mapping and linkage group identification. However, direct use of cytological markers has been very limited in genetic mapping and plant breeding.

Genetic diversity analysis in 28 guava genotypes of Brazil origin by morphological, karyotypical, nuclear 2C-value and simple sequence repeat revealed that the genotypes have a stable, very small diploid genome ($2n = 2x = 22$; $2C = 0.95$ pg) (Coser *et al.*, 2012).

Variation in ploidy was reported in many cultivars of *P. guajava* L in spite of diploid somatic chromosome number $2n = 2x = 22$. Previous cytological records on different species of *Psidium* suggests the existence of ploidy series with a basic chromosome number $x = 11$. However, somatic chromosome number recorded in the *P. guineense* clearly suggests the multiple of basic chromosome number $x = 11$

indicating polyploidy in nature. Two species, *P. molle* and *P. guineense* were found to be morphologically similar with minute differences in their chromatographic pattern. Close affinity between *P. guineense*, *P. pumilum* and *P. chinensis* was observed (Das and Prakash, 1981).

The extra chromosome was found to be transmitted through both the egg cell and the pollen. However, the frequency of transmission was greater through the egg cell than the pollen. As high as 26% transmission of extra chromosomes were obtained through the egg cell. There was no clear cut difference between trisomics and higher aneuploids with regard to the frequency of transmission of extra chromosome (Mohammed and Majumder, 1974).

Chromosome number and karyotype constancy recorded shows a stable genotypic nature of the species. The size variation of somatic chromosomal complements of *P. guineense* reveals a tendency to asymmetry. According to Stebbins (1971) the karyotype of this species belongs to the category 2A and the accumulation of relative size differences between the chromosomes of the complement suggests its karyotype to be considered as slightly asymmetric. Moderate degree of symmetry in *Psidium* and other members of Myrtaceae were also reported by Vijayakumar and Subramanian (1985). Therefore, the chromosome constancy recorded by Chakraborti *et al.* (2010) clearly reveals a new record of the wild guava (*P. guineense* Sw.) of somatic chromosome number $2n = 44$. The karyotype of the species was slightly asymmetric with a little variation in chromosome length. The species revealed 4 pairs of metacentric, 12 pairs of nearly metacentric and 6 pairs of sub-metacentric chromosomes one of which also had secondary constriction. No meiotic irregularities were recorded.

P. cattleianum var. *lucidum* was reported to be octaploid (as compared with *P. molle*, which was tetraploid and the other species, which were diploids of $2n = 22$) (Seth, 1963).

Seedless varieties were reportedly common in *P. guajava* as stated by Seth (1959). The seedless trait is related to many factors, of which self-incompatibility and chromosomal abnormalities were considered to be the major ones. The variety 'Seedless' was diploid with $n = 22$. Both embryo sac and pollen grains were found to be functional, but the percentage of viable pollen grains was low. The meiotic division was highly abnormal showing monovalent and bivalent laggards as well as bridging of chromosomes, indicating the hybrid origin of the variety and explaining the low pollen grain fertility.

The chromosome number of *P. guajava* was reported as $2n = 22$ (Soubihe Sobrinho, 1951; Kumar and Ranade, 1952), but the level of ploidy was verified. Kumar and Ranade (1952) mentioned a seedless variety found to have a somatic complement of 33 chromosomes, which appeared to be the first record of triploidy.

In this matter, Hirano (1967) reported the following results which was obtained in his study for chromosome counts: *P. cattleianum* ($2n = 77$; based on two specimens), *P. cattleianum* var. *lucidum* ($2n = 66$; based on one specimen); *P. cujavillus* ($2n = 44$); *P. guineense* ($2n = 44$); *P. friedrichsthalianum* ($2n = 44, 66$); *P. polycarpum* ($2n = 22$); *P. guajava* several cultivars ($2n = 21, 22, 24, 25, 33$).

Polyploidy within *P. guajava* was reportedly uncommon but the genus *Psidium* itself is represented by di-, tetra-, hexa- and octoploid species (Hirano and Nakasone, 1969). For *P. guajava* and *P. polycarpum* $2n = 22$; for *P. guineense* and *P. cujavillus* $2n = 44$; for *P. friedrichsthalianum* $2n = 66$. Although reports of $2n = 88$ have been made for the chromosome number of *P. cattleianum* and its botanical form *P. cattleianum* f. *lucidum*, two plants of *P. cattleianum* were found to be heptaploid with $2n = 77$ and three plants of *P. cattleianum* f. *lucidum* were hexaploid with $2n=66$ in this study. *P. guineense* and *P. cujavillus* were introduced into Hawaii as such, but similarities in chromosomes as well as in vegetative characters between these species casts some doubt as to their identities.

Pollen of *P. guajava* (cultivars used had $n = 22$ and $n = 33$) generally had high germination rates, which were higher than those for species with higher chromosome numbers. *P.*

cattleianum and *P. cattleianum* f. *lucidum*, with reports of $n=88$, were sometimes found to be heptaploid (*P. cattleianum*, $2n=77$) and hexaploid (*P. cattleianum* f. *lucidum*, $2n=66$) (Hirano and Nakasone, 1969).

2.11 Fruit morphological traits

In a study conducted to estimate the genetic relationship between 20 genotypes of *Psidium guajava* and two species, *P. friedrichsthalianum* Ndz. and *P. cattleianum* Sabine, out of 16 morphological characters studied including fruit shape, length, diameter, weight, skin and pulp colour, all traits showed variability to considerable extent except inflorescence type (Sharma *et al.*, 2010). Mahmoud and Peter (2014) reported that physical fruit characters of guava fruits varies significantly among different guava genotypes. There is a varietal variation for fruit weight, length, breadth, seed content, TSS and acidity (Singh and Moti, 1988).

The yield and quality traits of seven year old guava (*Psidium guajava*) hybrids, both red and white flesh types, were evaluated in Meghalaya, India. Hybrid-3 topped with respect to productivity (22.50 kg/tree). Fruit weight was maximum in Hybrid-7 (151.50 g) which was followed by Hybrid-11 (128.25 g) (Babu *et al.*, 2007).

Kumar (1998) has found the maximum fruit weight in cultivar Arka Mridula, while it was minimum in Red Fleshed. The fruit length also exhibited significant differences, while non-significant differences were obtained with respect to fruit breadth. Maximum fruit length (7.27 m) and breadth (7.43 cm) were in Arka Amulya and CISH-G-31, respectively, while these were minimum length (5.83 cm) and breadth (6.37 cm) were recorded in Lalit.

Screening was carried out on hundred guava genotypes in respect of fruit physical attributes during 2010-2011. Physical fruit characters of guava fruits vary significantly among different guava genotypes. Out of hundred guava genotypes, thirteen elite guava genotypes (genotype no 100,99,30,50,4,94,57,88,5,33,3,8 and 67) topped the rest of genotypes in term of physical characters. Tree no. 99 was found superior over all genotypes, in term of fruit weight (300.5g) fruit diameter (7.36cm), less number of seed per fruit (0), lowest seed weight (0.00g), lowest seed weight to fruit weight ratio (0%), higher pulp thickness (3.2cm), higher pulp weight (271.7g), pulp to fruit weight ratio (88.7%), maximum peel weight (28.83g) (Mahmoud and Peter *et al.*, 2014).

Sharma *et al.* (2010) studied variations in the fruit shape among the different genotypes studied, which varies from ovate, obovate, round, oblate, pear shaped, oblong, and oval.

A study conducted on 16 morphological characters on 20 genotypes of guava reported variations in the fruit peel colour among the different genotypes studied, which includes green, yellowish green, greenish yellow, green with reddish blush etc. (Sharma *et al.*, 2010).

According to Kidaha *et al.* (2015) reported the Kenyan guava exhibited morphological diversity among guava landraces of Western and Coastal Kenya. Ripe fruit color varied from pink to cream.

Mitra *et al.* (1983) evaluated eleven varieties of guava and found that the variety Sardar guava was superior to all varieties considering the yield, fruit size and fruit weight. Allahabad Safeda and Baruipur were also superior in pulp percentage, but the fruits were seedy. The fruits of variety Seedless had the least number of seeds, but the size of fruits was small.

Sehgal and Singh (1965) studied the performance of five varieties of guava viz., Safeda, Chittidar, Sardar, Red Fleshed and Seedless and found that the fruits of Sardar had maximum weight of seeds per fruit. Sardar guava was found most promising under Delhi condition on the basis of flesh colour, seedlessness, skin colour, flavour, fruit shape and fruit surface.

Kotalawala (1971) studied the performance of the four varieties of guava viz. Seedless, Safeda, Wickramasekara and Allahabad in the intermediate zone of ceylone with a shallow soil and an annual rainfall of about 55 inches. Safeda was the best in terms of yield, total number of fruits and average weight per fruit.

2.12 Fruit biochemical traits

Pandey *et al.* (2007) TSS was highest (15.67 °B) in Hisar Surkha, whereas it was lowest (11.27 °B) in Hybrid-21. Similarly, acidity (0.15%) was lowest in cv. Hisar Surkha and highest acidity (0.36%) in cv. Sangam. The ascorbic acid content ranged from 104.17 to 243.75 (mg 100g⁻¹ pulp) among the different cultivars with maximum in Shweta. In general, higher ascorbic acid content (200 mg 100g⁻¹ pulp or above) was recorded in Arka Amulya, Hybrid-21, Sangam and Hisar Safeda.

The strawberry guava and lemon guava fruits are typically wild-harvested and mainly consumed as juice, jellies and confectionery. These fruits are a source of natural antioxidants and antimicrobial and antiproliferative agents. They are often used for the treatment of diseases like diarrhoea (De Souza *et al.*, 2004; Medina *et al.*, 2011).

Shrivastava and Shrivastava (1965) described two important commercial guava varieties of India viz. Allahabad Safeda (White Fleshed) and Red Fleshed. Allahabad Safeda fruits had maximum value of T.S.S., pH, acidity, ascorbic acid and sugars, whereas Red Fleshed was found rich in starch, crude fiber and tannin content.

Singh and Moti (1988) also observed lowest ascorbic acid content in some cultivars in the central gangtic plains. Total sugar content was highest (9.32%) and lowest (6.67%) in Hisar Surkha and Hisar Safeda, respectively.

Roy and Ahmed (1951) studied the performance of four varieties of guava viz. Harijha, Safeda, Hafsi and Seedless in Bihar. They found that the Hafsi had minimum fruit weight and Harijha gave maximum yield. Variety Seedless was slightly higher in vitamin C content than others. Fruits of variety Safeda were superior in quality, but numerous hard seeds, Harijha had fewer seeds but were inferior in taste and flavour as compared to Safeda.

Teaotia *et al.* (1968) described the ten Red Fleshed varieties of guava and reported that Red-fleshed Allahabad had the maximum fruit weight (180.0g) followed by Spear acid (134.0g) whereas China Surkha had the minimum fruit weight (6.5g). The highest value of total sugar was recorded in China Surkha (10.68%). The maximum acidity was recorded in Patilo and the minimum acidity was found in Anakapalle. The T.S.S. content ranged from 9.27 to 17.45°Brix in variety Patilo and Arica Florida, respectively.

A study conducted by Vasugi and Dinesh (2007) reported that TSS for cultivar and wild species of guava was found to be ranged from 7.46 to 11.6°B in *P. cattleianum* var. *lucidum* and *P. molle* respectively and titratable acidity 0.6 to 2.7% in *P. chinensis* and *P. quadrangularis*.

In a study conducted by Vasugi and Dinesh (2007) on genetic variability in some *Psidium* species where they have reported the vitamin C content to be varied from 16.78 to 231.5 mg 100⁻¹ in *P. cattleianum* var. *lucidum* and *P. chinensis* respectively. Moreover, a profound effect on vitamin C content based on the different stages of maturation and geographical location was recorded by Gull *et al.* (2012).

Mowlah and Itoo (1982) reported that, in white and pink seedling varieties of guava, the main sugar components were fructose and sucrose. These are good with respect to the health point of view.

A study conducted by Jain *et al.* (2003) on the biochemistry of fruit ripening of guava (*Psidium guajava* L.): compositional and enzymatic changes, recorded total carotenoid of guava cultivar Banarsi Surkha as 0.42 mg 100 g⁻¹.

The yield and quality traits of seven year old guava (*Psidium guajava*) hybrids, both red and white flesh types, were evaluated in Meghalaya, India. Vitamin-C content was highest in Hybrid-3 (258.50 mg/100 g) followed by Hybrid-1 (240.00 mg/100 g). Total soluble solids content was highest in Hybrid-7 (11.5%) followed by Hybrid-1 (11.2%). The white flesh types, Hybrid-7 and Hybrid-1, with high TSS, vitamin-C and less pronouncement towards seediness are best suitable for growing under mid hill altitude of Meghalaya (Babu *et al.*, 2007).

2.13 Volatile aroma profiling

To overcome the limitations of morphological traits, other markers have been developed at both the protein level (phenotype) and the DNA level (genotype). Protein markers are usually named 'biochemical markers'. Protein markers (seed storage proteins and isozymes) are generated through electrophoresis, taking advantage of the migrational properties of proteins and enzymes, and revealed by histochemical stains specific to the enzymes being assayed. Isozymes reflect the products of different alleles rather than different genes because the difference in electrophoretic mobility is caused by point mutation as a result of amino acid substitution (Xu, 2010). Therefore, isozyme markers can be genetically mapped onto chromosomes and then used as genetic markers to map other genes. They are also used in seed purity test and occasionally in plant breeding. However, protein markers are also limited by being influenced by the environment and changes in different developmental stages. There are only a small number of isozymes in most crop species and some of them can be identified only with a specific strain. Therefore, the use of enzyme markers is limited.

Great deal of attention has been directed towards the characterisation of the volatile profile of a wide variety of exotic fruits over the past several decades (Lasekan and Abbas, 2012).

The first study on the volatile profile of red and yellow guava fruits was carried out in Japan (Shiota *et al.*, 1980). After that, Vernin *et al.* (1998) reported on the identification of numerous volatile compounds from guava fruits cultivated on Reunion Island. More recently, a report on the chemical composition comparison between yellow and red guava fruits from Brazil has shown that b-caryophyllene is the main component in the essential oils of both fruits (Bieglmeyer *et al.*, 2011).

According to Egea *et al.* (2014) the most powerful odorant in the guava fruits was (Z)-3-hexenal with 84% and 82% in the lemon guava and strawberry guava, respectively. This aldehyde is formed by enzymatic oxidation of linoleic acid during cell breaking and it has a characteristic green leaf odour. Among the quantified aldehydes, (E)-2-hexenal was the most abundant, at more than 100g kg⁻¹ in both lemon guava and strawberry guava. In addition, (Z)-3-hexenal and hexanal were also released in significant amounts, with larger quantities in the strawberry guava pulp.

The compound (Z)-3-Hexenal has been demonstrated to be a predominant compound in the aroma of fruits, such as pink guava (Steinhaus *et al.*, 2009), Japanese lemon and strawberry guavas (Vernin *et al.*, 1998).

Pino (2007) characterized the volatile compounds in different varieties of guava from Columbia and identified ninety-seven compounds of which 19 were identified for the first time as volatile constituents of guava. Each variety has a typical composition, characterized by a specific ratio between the main components and classes of substances.

Soares *et al.* (2007) studied the effect of the maturation stages on the chemical composition and volatile compounds of the white guava (*Psidium guajava*) cv. Cortibel were investigated during three

different stages. The stages were characterized by evolution of color, pH, titratable acidity, sugars, soluble solids, vitamin C and volatile components. The fruits were stored at 24°C and air humidity of 74% for 13 days. The volatile extracts were obtained using headspace technique and analyzed using gas chromatograph/mass spectrometry (GC/MS) system. The titratable acidity and sugars decreased. The pH level and amount of vitamin C increased throughout the process of maturation. The behavior of volatile compounds of fruits in the three stages of maturation was: in immature fruits and those in their intermediate stage of maturation, were predominantly the aldehydes such as (E)-2-hexenal and (Z)-3-hexenal. In mature fruits, esters like Z-3-hexenyl acetate and E-3-hexenyl acetate and sesquiterpenes caryophyllene, α -humulene and β -bisabolene are present.

A total of sixty five compounds were identified from Guava cv. Chung-Shan-Yueh-Pa by using capillary GC-MS and sensorily characterized by GC-sniffing. α -pinene, 1, 8-cineole, β -caryophyllene, nerolidol, globulol, C6 aldehydes, C6 alcohols, ethyl hexanoate and (Z)-3-hexenyl acetate were the major constituents identified. The unique flavour of the guava fruit is due to the presence of C6 aldehydes, C6 alcohols, ethyl hexanoate, (Z)-3-hexenyl acetate, terpenes and 1, 8-cineole. In this study γ -bisabolene and zingiberene were also identified for the first time in the guava fruit (Chen *et al.*, 2006).

Terpene hydrocarbons were identified in guava puree and β -caryophyllene reported to be the major compound which imparts aroma (Wilson and Shaw, 1978).

In a study conducted on strawberry and lemon guavas, 24 odour zones were detected by gas chromatography–olfactometry (GC-O) among which 16 and 17 zones were found in extracts of strawberry and lemon guava pulp respectively. Ten compounds were common in both strawberry and lemon guavas. Guava fruits are having the typical aroma due to the presence of aldehydes and ketones. (Z)-3-hexenal was the prominent odorant present in the samples. Other compounds like 1,8-cineole and linalool were also revealed as important aroma constituents (Egea *et al.*, 2014).

McLeod and Troconis (1982) analysed the volatile flavour components of guava using GC/MS of both EI and CI techniques. They found that the esters comprised over 55% of the essence and an extensive series of ethyl esters of the even carbon-numbered carboxylic acids was observed (over 52% of the sample) among which two monoterpene hydrocarbons and five sesquiterpene hydrocarbons.

Phylogenetic studies carried out in *Psidium species* utilizing differences in flavonoid patterns showed a close affinity between *P. guajava* and *P. molle* (Das and Prakash, 1981).

In a study conducted by Shibamoto and Tang (1990) and Olle *et al.* (1997) on various mango cultivars, it has been reported that more than 285 volatile compounds are present which include monoterpenes, sesquiterpenes, esters, aldehydes, ketones, alcohols, acids, aliphatic hydrocarbons and aromatics. In another study conducted by Winterhalter (1991) revealed that 70-90% of total volatiles present in mango cultivars is contributed by hydrocarbons, monoterpenes and sesquiterpenes.

In a study conducted on volatile analysis of fruit juice of different citrus species, the major terpene component in Dordoir orange juice was reported to be L-limonene (Mas *et al.*, 2011). In a study conducted in Mango (*Mangifera indica* L.) by the application of distillation extraction in combination with active odor value (aroma threshold) technologies exhibited that monoterpenes such as α -pinene was found to be highest (Pino and Mesa, 2006).

In a study conducted by Chen *et al.* (2006) on *Psidium guajava* L. cv. Chung-Shan-Yueh-Pa, β -caryophyllene was found to be the major compound contributing to aroma of guava. In another study conducted on guava puree by Wilson and Shaw (1978) reported that β -caryophyllene is the major aroma contributing compound in guava.

In a study conducted by Egea *et al.* (2014) on the comparative analysis of aroma compounds and sensorial features of strawberry and lemon guavas (*Psidium cattleianum* Sabine), they have reported that the (Z)-3-hexenal is the major compound which gives aroma to guava fruits.

A study conducted on identification of volatile components from mango (*Mangifera indica* L.) cultivar conducted by Pino and Mesa (2006) reported that caryophyllene oxide as a compound contributing to aroma of mango.

In a study conducted in Colombian mango, the major volatile present in Hilacha” and “Vallenato” varieties were reported to be α -pinene (Olle *et al.*, 1998).

In a study conducted by Chen *et al.* (2006) on *Psidium guajava* L. cv. Chung-Shan-Yueh-Pa, that in guava fruits, sesquiterpene alcohols amounted nearly 21% of the total volatiles, and included nerolidol as a major compound. In a study conducted by Paniandy *et al.* (2000) nerolidol was reported as the major volatile constituents in the guava fruit.

In a study conducted on determination of aroma compounds in blackberry by GC/MS analysis by Turemis *et al.* (2003) carvone have been identified as a major volatile compound. It has been reported in a study conducted in mango by Pino and Mesa *et al.* (2006) that (*E*)- β -ionone as one of the most important compound contributing to mango flavor.

III. MATERIALS AND METHODS

The present investigation on “Studies on utilization of wild species of guava (*Psidium guajava* L.) for crop improvement” was carried out during 2016-2017 at the Division of Fruit crops, ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru. The details of materials used and methodology adopted during the course of investigation are given below.

3.1 Location

The field and laboratory experiments were carried out at Indian Institute of Horticultural Research, Hessarghatta Lake Post, Bengaluru located at 13° 58' north latitude and 78° east longitudes and at an altitude of 890 m above mean sea level. Soil is red sandy loam with a pH of 5.2-6.4.

3.2 Weather and climate

The climate of Hessarghatta is moderately warm with mild summer months. The maximum mean temperature ranges from 25.2 °C to 31.7°C with a mean of 27.93°C, while the minimum mean temperature ranges from 15.1°C to 21.7°C with a mean of 18.43°C. The mean relative humidity, mean wind speed and total rainfall was 68.38 per cent, 2.53km hr⁻¹ and 336.46 mm respectively for the month of July 2016 to May 2017 (Annexure I).

3.3 Materials

The present investigations were carried out as 1) Studies on phenology of wild species and its crossability with *Psidium guajava* L. varieties 2) Cytological studies on *Psidium guajava* varieties and their wild species 3) Morphological and biochemical traits and volatile aroma profiling of fruits of *Psidium guajava* varieties and their wild species.

The wild species (*P. guineense*, *P. chinensis*, *P. cattleianum*) and *P. guajava* L. varieties (Arka Kiran, Arka Rashmi, Arka Mridula, Arka Amulya, Allahabad Safeda, Purple Local, Thailand guava, Seedless Triploid and Local Red) collected and maintained in the Field Gene Bank of ICAR-IIHR were used in the study. These accessions were planted at a spacing of 5 × 5 m with three plants per accession representing individual tree as one replication (Plate 1.). These trees were of 6 to 7 years of age and were maintained by following the standard package of practices.



Plate 1. Field view of the experimental plot

3.3.1 Studies on phenology of wild species and its crossability with *Psidium guajava* L. varieties

The brief description of guava varieties and wild species used in the study is as follows;

***P. guineense* Sw.** (Syn: *P. araca* Raddi, *P. molle* Bertol, *P. monticola* O. Berg, *P. schedianum* O. Berg):

It is commonly called as Brazilian guava or Castilian guava or Guinea guava. The flavour is acidic. It is reported to have drought hardy nature. Singh *et al.* (1976) reported that it is resistant to wilt.

***P. chinensis*:**

The plants are medium tall with narrowly elliptic leaves. The fruits are small, globose with light yellow pericarp on maturity. Pulp is firm and sweet with a TSS of 8.0 to 8.5 °B. Fruit consist of small medium soft seeds, rich in vitamin C content (231.5mg 100 g⁻¹ pulp). The average titrable acidity is 0.6 % and total sugar is 9.34 g 100g⁻¹ pulp (Vasugi and Dinesh, 2007).

***P. cattleianum* Afzel. ex Sabine** (syn: *Psidium cattleianum* var. *longipus* (O.Berg) Fosberg, *P. cattleianum* var. *littorale* Raddi, *P. littorale* Raddi, *P. cattleianum* var. *lucidum* S. :

It is commonly called as cattley guava, cherry guava, purple guava, red strawberry guava and strawberry guava. Fruits are small, round, red pericarp with white soft and juicy flesh contains several hard seeds. It has sweet flavor and good aroma. It is cold hardy (Julian, 1998) species. It is also reported to be resistant to wilt (Leslie *et al.*, 1995 and Edward and Shankar, 1964). Singh *et al.* (1977) reported that high yields were obtained while using *P. cattleianum* as rootstock.

Arka Kiran:

It is selected from the cross 'Kamsari' × 'Purple Local'. Plants are semi vigorous, amenable for high density planting. The fruits are sub globose, weighing about 180-200 g. The pulp is deep red with good flavour, high lycopene content (6.0 to 7.0 mg 100g⁻¹), TSS of 12.0 to 12.5°B with medium seed hardness (9.0 kg cm⁻²).

Arka Rashmi:

It is selected from the cross 'Kamsari' × 'Purple Local'. Dual purpose variety with good vitamin C content (180 to 200 mg 100g⁻¹) and medium in lycopene (5.0 mg 100 g⁻¹) and thick pulp, medium size round fruits , red pulp with low oxalate content (28.3 mg 100 g⁻¹ FW), and medium seed hardness (9.0 to 10.0 kg cm⁻²) with a TSS of 11 to 12°B .

Arka Mridula:

It is a selection from Allahabad Safeda with medium sized fruit (180 to 200g) having soft seeds. The pulp is white in colour with a TSS of 12°B.

3.3.2 Cytological studies of *P. guajava* varieties and wild species of guava

The root tips of freshly sprouted healthy *P. guajava* varieties (viz., Arka Kiran, Arka Rashmi, Arka Mridula, Arka Amulya, Seedless triploid, Purple guava, Thailand guava and Local Red) and wild species viz., *P. guineense*, *P. cattelianum* and *P. chinensis* were used in the study. A brief description of guava varieties and wild species used in the study is as follows;

Arka Amulya:

It is selected from the cross 'Allahabad Safeda' × 'Seedless Triploid'. The plants are semi-vigorous and spreading type. Fruits are medium sized (180-200g), white pulp with a high TSS of 12.5°B.

Local red:

The fruits are sub- globose shaped and small to medium in size (100 g) with TSS ranging from 7.0 to 8.0 °B.

Purple Local:

The pericarp is purple with pale pink pulp. The fruits are small with soft seeds and medium in taste.

Seedless triploid:

The plants are very vigorous. Fruits are of irregular shape and with thin skin, warty surface and swollen calyx end. Pulp is white, contains few seeds and has good taste and aroma.

Thailand guava:

The fruits are sub- globose, medium in size (160 g) with pale pink pulp and TSS (8.0 to 9.0 °B).

3.3.3 Morphological and biochemical traits and volatile aroma profile of *P. guajava* L. varieties and wild species of guava

The fresh aromatic (colour break stage) fruits of *P. guajava* varieties (Arka Mridula, Arka Kiran, Arka Rashmi, Allahabad Safeda, Arka Amulya, Purple local, Seedless triploid and Local Red) and wild species (*P. guineense*, *P. cattelianum* and *P. chinensis*) of guava were used for volatile aroma profiling. A brief description of guava varieties and wild species used in the study is as follows;

Allahabad Safeda:

It is the most popular variety in India and is the progenitor of many Indian varieties and occupies the largest area under cultivation. Fruits are round, medium to large in size, smooth skin, pulp white, firm, excellent in quality with high TSS and vitamin C, pleasant flavour and a few soft seeds.

3.4 Methodology

3.4.1 To study the phenology of wild species and its crossability with *P. guajava* L. varieties

The design, treatment details and the procedure followed with respect to phenology and crossability are detailed as follows:

3.4.1.1 Design

For phenological studies, row trial was followed, where three trees were planted for each accession. For crossability study, Randomized Complete Block Design (RCBD) was used.

3.4.1.2 Treatment details

3.4.1.2.1 Phenological study

Number of Treatments: Four

Number of Replications: Three

Number of branches per replication: Ten

T₁: *Psidium guineense*

T₂: *Psidium chinensis*

T₃: *Psidium cattleianum*

T₄: *Psidium guajava* var. Arka Kiran

3.4.1.2.2 Crossability study

The field view of the female and male parents used in the study are given in the plates 2, 3, 4, 5, 6.

Number of Treatments: Twelve

Number of Replications: Three

T₁: Arka Kiran × *Psidium guineense* (control)

T₂: Arka Kiran × *Psidium guineense* (nutrient solution)

T₃: Arka Mridula × *Psidium guineense* (control)

T₄: Arka Mridula × *Psidium guineense* (nutrient solution)

T₅: Arka Rashmi × *Psidium guineense* (control)

T₆: Arka Rashmi × *Psidium guineense* (nutrient solution)

T₇: Arka Kiran × *Psidium chinensis* (control)

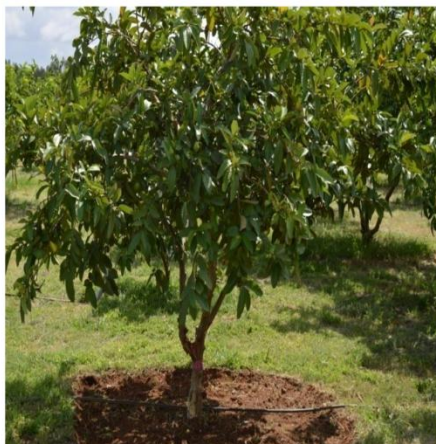
T₈: Arka Kiran × *Psidium chinensis* (nutrient solution)

T₉: Arka Mridula × *Psidium chinensis* (control)

T₁₀: Arka Mridula × *Psidium chinensis* (nutrient solution)

T₁₁: Arka Rashmi × *Psidium chinensis* (control)

T₁₂: Arka Rashmi × *Psidium chinensis* (nutrient solution)



a) Fully grown tree



b) Fully opened flower

Plate 2. Field view of female parent : Arka Kiran used in interspecific hybridization



a) Fully grown tree

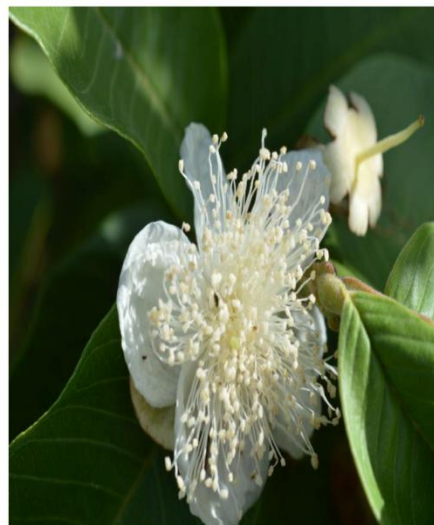


b) Fully opened flower

Plate 3. Field view of female parent : Arka Mridula used in interspecific hybridization



a) Fully grown tree



b) Fully opened flower

Plate 4. Field view of female parent : Arka Rashmi used in interspecific hybridization



a) Fully grown tree



b) Fully opened flower

Plate 5. Field view of male parent: *Psidium guineense* used in interspecific hybridization



a) Fully grown tree



b) Fully opened flower

Plate 6. Field view of male parent: *Psidium chinensis* used in interspecific hybridization

3.4.1.3 Procedure

In the phenological study, ten branches from each tree were selected from each accession to record the observations and the mean value was worked out and taken for basic statistical analysis.

The interspecific hybridization was carried out using *P. guajava* varieties viz., Arka Kiran, Arka Mridula and Arka Rashmi as female parents and *P. guineense*, *P. chinensis* as male parents.

Pollen from freshly opened, already selected and bagged male parents viz., *P. guineense* and *P. chinensis* were collected and dusted onto the already selected, emasculated and bagged female flowers either directly on stigma (in control) or by making pollen slurry with 5% nutrient solution, which consists of sucrose 5% + Boric acid 100 ppm + MgSO_4 200 ppm + CaNO_3 300 ppm + KNO_3 100 ppm. After pollination, flowers were labelled and then covered using butter paper. The flower buds which are about to open the next day (calyx break stage) were selected and covered using butter paper bags and used as pollen parent. In female parents, the selected female flowers were emasculated using sharp pointed forceps leaving the stigmatic surface intact and then were covered using butter bags for pollination on the next day (Plate 7).

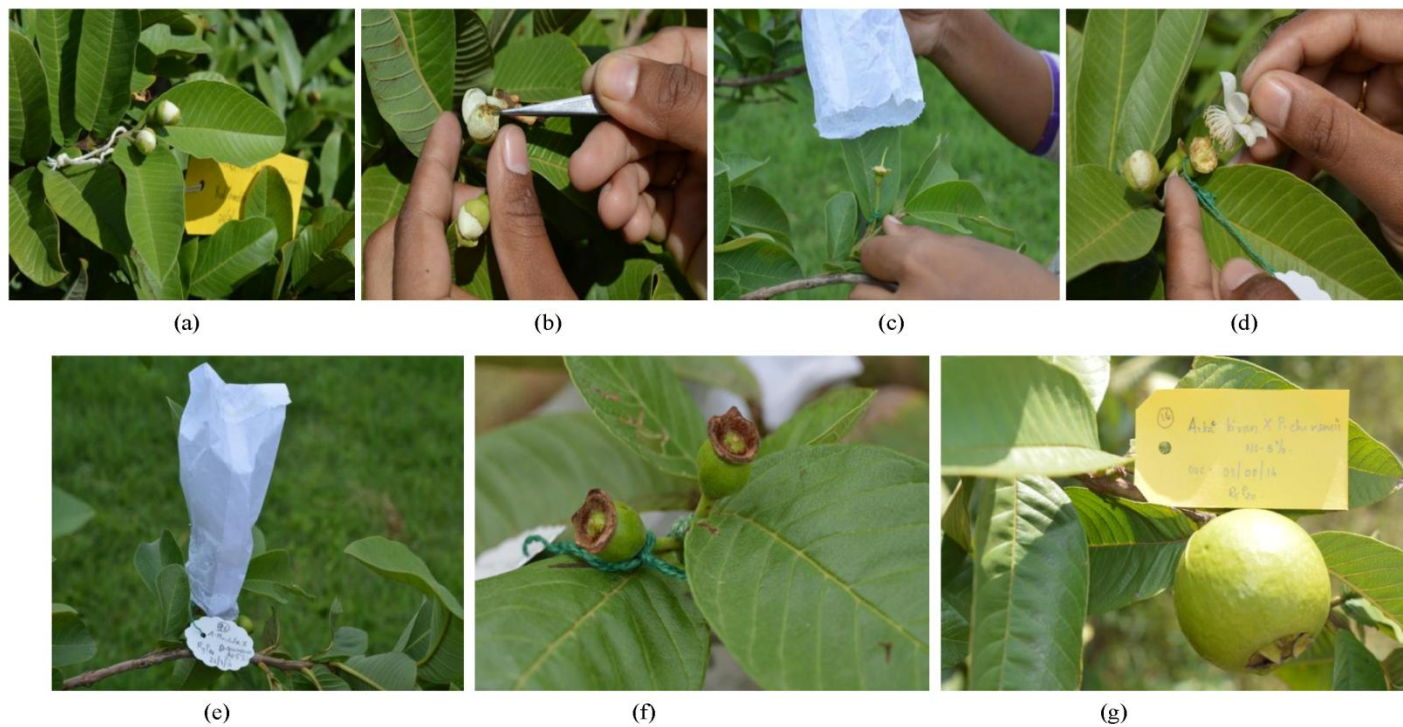


Plate 7. Steps involved in interspecific hybridization

(a) Flower at calyx break stage, (b) Emasculation of female flower, (c) Bagging of emasculated flower, (d) crossing with the male flower, (e) Rebagging after crossing, (f) Fruit set after 15 days, (g) Fruit at harvest stage

3.4.1.4 Observations recorded

The observations pertaining to the phenological study (3.4.1.4.1 to 3.4.1.4.10) and with respect to crossability study (3.4.1.4.11 to 3.4.1.4.15) are as follows;

3.4.1.4.1 Time of leaf flushing

Days taken from leaf bud emergence, when the leaf bud becomes visible on the newly emerged shoot to the leaf emergence was calculated and expressed in number.

3.4.1.4.2 Time taken from flower bud initiation to full bloom

Days taken from flower bud initiation (when the flower bud becomes visible in the axils of leaves) to full bloom was counted and expressed in number.

3.4.1.4.3 Time of flowering

The month in which flowering was noticed among different species was recorded.

3.4.1.4.4 Duration of flowering

The days taken by each plant from occurrence of first flowering to last flowering was counted and expressed in number.

3.4.1.4.5 Peak flowering

Days taken to 50 per cent flowering in each tree was counted from the start of flowering and expressed in days.

3.4.1.4.6 Time of anther dehiscence

The time at which anther dehiscence was observed in different species was recorded and expressed in hours.

3.4.1.4.7 Pollen viability assessment

The per cent germination of pollen using freshly opened flowers was calculated by adopting hanging drop method.

3.4.1.4.8 Time taken from flowering to fruit set

The days taken from anthesis to development of peanut stage fruit was counted and expressed in number.

3.4.1.4.9 Time taken from fruit set to harvest

The days taken from fruit set (pea size) till it reaches horticultural maturity was counted and expressed in number.

3.4.1.4.10 Duration of fruiting

The days taken from first fruiting to last fruiting by each tree was counted and expressed in number.

3.4.1.4.11 Number of flowers crossed

The total number of flowers crossed per treatment was counted and expressed in number.

3.4.1.4.12 Per cent fruit set

The number of fruits which developed and reached pea stage after two weeks of pollination was counted and was expressed as per cent.

3.4.1.4.13 Number of fruits harvested

Fruits that are retained till the mature stage (colour break stage) were harvested and total number of harvested fruits was obtained by counting the fruits.

3.4.1.4.14 Number of seeds per fruits

The number of seeds obtained per fruit was counted and expressed in number.

3.4.1.4.15 Per cent germination

The number of seedlings emerged from each treatment consisting of hundred seeds was counted after three weeks of sowing and was expressed as per cent.

3.4.1.5 Statistical methods

For phenological studies, the basic statistical measures were worked out. The data on crossability studies were subjected to Fisher's method of analysis of variance (ANOVA) as outlined by Sundarraj *et al.* (1972). Wherever the 'F' test was significant for comparison of treatment means, CD values were worked out at 0.05 probability level.

3.4.2 Cytological studies on *P. guajava* varieties and their wild species

The chromosome number or ploidy was carried out using root tips obtained from young sprouts of *P. guajava* varieties (Arka Amulya, Arka Kiran, Arka Mridula, Arka Rashmi, Local Red, Purple guava, Seedless triploid and Thailand Guava) and the wild species (*P. cattelianum*, *P. chinensis* and *P. guineense*).

3.4.2.1 Treatment details

Number of Treatments: Eleven

- T₁: Arka Amulya
- T₂: Arka Kiran
- T₃: Arka Mridula
- T₄: Arka Rashmi
- T₅: Local Red
- T₆: *P. cattelianum*

T₇: *P. chinensis*
T₈: *P. guineense*
T₉: Purple local
T₁₀: Seedless triploid
T₁₁: Thailand guava

3.4.2.2 Procedure

Root tips of *P. guajava* varieties and wild species were collected from young sprouts and rinsed in distilled water. Young root tips measuring 2-3 mm was pretreated with 0.003M 8 Hydroxyquinone for 2 hours at 14-16°C. It was then rinsed in distilled water and then was fixed in Carnoy's-II fixative viz., 6:3:1 of absolute alcohol: glacial acetic acid: chloroform and was stored for 24 hours. Later they were transferred to 70% alcohol after 24 hours of fixation for long term storage (about 2-4 months). The stored root tips were then rinsed in distilled water and hydrolysed in a water bath with 1N HCL at 60°C for 5 minutes or in 5N HCL at room temperature for 30 minutes. The hydrolysed root tips were then transferred to Schiff's reagent (Lillie,1951) also known as Feulgen stain after rinsing in distilled water, and were stored in dark for 1½ to 2 hours. Schiff's reagent will stain the actively dividing meristematic tissue to deep magenta colour. The stained tips were squashed with a drop 1% aceto-carmin or orcein after removing the root cap. The slides were sealed with wax or nail polish and observed under microscope in the same day or the next day. The slides were scanned for the well spread metaphase chromosomes and selected for counting the number of chromosomes (Hiremath and Chennaveeraiah, 1982). Ten to fifteen such cells were selected and counted.

3.4.2.3 Observation recorded

Chromosome number/ploidy was recorded by counting the number of chromosomes in the metaphase.

3.4.3 Morphological and biochemical traits and volatile aroma profiling of fruits of *Psidium guajava* L. varieties and the wild species

The morphological and biochemical traits and volatile aroma profiling of fruits of *P. guajava* L. varieties and the wild species were carried out using the fresh fruits which were at colour break stage.

3.4.3.1 Design

Completely Randomized Design (CRD) was used for morphological and biochemical traits.

3.4.3.2 Treatment details

Number of Treatments: Eleven

Number of Replications: Three

Treatment details:

T₁: Allahabad Safeda
T₂: Arka Amulya
T₃: Arka Kiran
T₄: Arka Mridula
T₅: Arka Rashmi
T₆: Local Red
T₇: *P. cattelianum*
T₈: *P. chinensis*
T₉: *P. guineense*

T₁₀: Purple local
T₁₁: Seedless triploid

3.4.3.3 Procedure

3.4.3.3.1 Morphological traits

The morphological traits like fruit weight, volume, length, width, thickness of outer pulp in relation to core diameter, fruit shape, pericarp/peel colour, core diameter, hundred seed weight, seed number, seed hardness and pulp colour was carried out using randomly selected fresh fruits at colour break stage. The methodology as detailed in DUS descriptor was followed for morphological traits.

3.4.3.3.1.1 Fruit weight

The weight of ten randomly selected fruits were recorded individually and was expressed in grams.

3.4.3.3.1.2 Fruit volume

Fruit volume of individual fruits was measured using water displacement method and was expressed in milliliters.

3.4.3.3.1.3 Fruit length

Fruit length was measured from the calyx end to the tip of the longitudinally cut open fruits using a measuring scale. It was then expressed in centimeters.

3.4.3.3.1.4 Fruit width

Fruit width was measured on a longitudinally cut open fruit at its broadest portion using a measuring scale. It was then expressed in centimeters.

3.4.3.3.1.5 Thickness of outer pulp in relation to core diameter

The thickness of the fruit leaving the core portion or the flesh embedded with seeds was measured using a measuring scale and was expressed in centimeters.

3.4.3.3.1.6 Fruit shape

Shape of ten randomly selected fruits in each treatment was recorded by visual observation.

3.4.3.3.1.7 Pericarp/peel colour

Pericarp or peel colour of ten randomly selected fruits per each treatment was recorded by visual observation

3.4.3.3.1.8 Core diameter

The core portion of the fruit was measured leaving the rind using a measuring scale and was expressed in centimeters.

3.4.3.3.1.9 Hundred seed weight

Hundred seeds were counted and weighed and value obtained was expressed in grams.

3.4.3.3.1.10 Seed number

The average number of seeds per fruit was recorded from ten randomly selected fruits per treatment.

3.4.3.3.1.11 Seed hardness

The hardness of seeds selected randomly from fruits collected was measured using seed hardness meter and was expressed in kg cm^{-2} .

3.4.3.3.1.12 Pulp colour

The colour of ten randomly selected fruits per treatment was recorded by using Royal Horticultural Society (RHS) colour chart. The observation was taken when fruits were at colour turning stage.

3.4.3.3.2 Biochemical traits

3.4.3.3.2.1 Total soluble solids

The total soluble solid of the fruit juice was determined using 'ERMA' hand refractrometer and was expressed in $^{\circ}\text{Brix}$.

3.4.3.3.2.2 Titrable acidity

Titration acidity was determined by using titration method and expressed as per centage of citric equivalents (AOAC, 2000).

Ten grams of fruit pulp was mixed with distilled water, squeezed through a muslin cloth and volume was made up to 50 ml. A known volume of the filtrate (10 ml) was titrated against 0.01 N NaOH using phenolphthalein as indicator. Titrable acidity was calculated as percentage of citric acid equivalents using citric acid standard curve.

3.4.3.3.2.3 Ascorbic acid

Ascorbic acid (Vitamin C) content was determined by 2, 6- Dichlorophenol indophenols (DCPIP) method and expressed as mg of ascorbic acid per 100g fresh weight (AOAC, 2006).

Ten grams of crushed fruit pulp was mixed thoroughly with 4% oxalic acid solution, squeezed through a muslin cloth and volume was made up to 50 ml. Vitamin C content present in the solution was estimated by titrating a known quantity of the extract against DCPIP and calculated as mg of ascorbic acid per 100 g fresh weight using a standard curve of L-ascorbic acid.

3.4.3.3.2.4 Total sugars:

The method suggested by Somogyi (1952) was used to estimate the total sugars and was expressed as g 100g⁻¹ FW.

One hundred milligram of the sample was weighed and extracted with hot 80% ethanol twice (5 ml each time). Supernatant was collected and evaporated by keeping it on a water bath at 80°C. Sugars were then dissolved by adding 10 ml water. Aliquot of 0.1 was pipetted out to separate test tubes. 0.2, 0.4, 0.6, 0.8, and 1 ml of the working standard solution was pipetted out to a series of test tubes. Volume was made up to 1 ml in both sample and standard tubes with distilled water. One ml of distilled water was pipetted out in a test tube as a blank. 1 ml of alkaline copper tartarate reagent was added to each test tube. The tubes was then placed in boiling water for 10 minutes, cooled and 1 ml of arsenomolybolic acid reagent was added to all the tubes. Volume of each test tube was made up to 10 ml with water and absorbance was read at 620 nm after 10 minutes. From the graph drawn, amount of reducing sugars present in the sample was calculated.

3.4.3.3.2.5 Lycopene

Total lycopene was estimated by spectrophotometric method which was expressed in mg 100g⁻¹ FW (Lichtenthaler, 1987).

Five grams sample was taken into a mortar, one spatula of CaCO₃ was added to this and ground with acetone. The residue was extracted repeatedly until the supernatant becomes colourless. All the extraction was carried out under low light or red light. The extract was taken in a separating funnel and 15 ml hexane and 100 ml water was added. The content was shaken well and was allowed to stand for a few minutes. The two phases formed was separated and the lower aqueous phase was re-extracted with additional hexane, until the aqueous phase become colourless and was dried with anhydrous Na₂SO₄ and the volume was made up to 25 ml with hexane and absorbance was read at 503 nm. Lycopene content was calculated and expressed as mg 100g⁻¹ fresh weight using standard curve.

$$\text{Lycopene (mg 100g}^{-1}\text{)} = \frac{\text{OD(503nm)} \times \text{Std. value (}\mu\text{g/OD)} \times \text{Total Vol. of extract} \times 100}{\text{Wt. of the sample (g)} \times 1000}$$

3.4.3.3.3 Extraction of volatile aroma compounds

A known quantity of the fresh aromatic fruit was homogenized for 1 min at 4°C and the homogenate was diluted with distilled water (1:4) and 0.5g of NaCl was added before extraction of aromas; the mixture was then incubated at room temperature with constant stirring on a magnetic stirrer (Shivashankara *et al.*, 2013). The sample was then placed in headspace vial (10mL volume) and capped. The SPME needle was pierced through the septum, and the plunger was depressed to expose the fibre to the headspace region of the sample vial. The fibre was exposed to the optimum headspace sampling condition of heating at 60°C for 60 min. The trapped volatile was desorbed at 250°C (2 min) in the GC injection port and flushed into the GC column and gas chromatography / mass spectrometry (GC/MS) was performed (Mazida *et al.*, 2003).

3.4.3.4 Observations recorded

The morphological traits like fruit weight, volume, length, width, thickness of outer pulp in relation to core diameter, fruit shape, pericarp/peel colour, core diameter, hundred seed weight, seed number, seed hardness and pulp colour were recorded.

The biochemical traits like TSS, titrable acidity, ascorbic acid, total, reducing and non reducing sugars, total carotenoids and lycopene content were recorded.

The major compounds contributing to the aroma of guava varieties and wild species was recorded.

3.4.3.5 Statistical methods

Morphological and biochemical traits data were subjected to Fisher's method of analysis of variance (ANOVA) as outlined by Sundarraj *et al.* (1972). CD values at 0.05 probability level were worked out when 'F' test was significant, for comparison of treatment means. Cluster analysis using Euclidean distance was carried out as per Ward's method to classify the varieties and species based on their similarities of the major aroma compounds (42 compounds which contributes to more one per cent of total aroma) using the XLSTAT software. The principal component analysis (PCA) is one of the multivariate methods that are applied to understand the variable leadings and to arrive at the most important variables which contribute to the diversity. PCA was also performed using XLSTAT software with the same aroma compounds which were selected for cluster analysis.

IV. EXPERIMENTAL RESULTS

The results of experiments on “Studies on utilization of wild species of guava (*Psidium guajava* L.) for crop improvement” carried out during 2016-2017 at the Division of Fruit crops, ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru are presented under the titles; Phenological stages of wild species, interspecific hybridization, cytological studies, fruit morphological traits, biochemical traits and volatile aroma profiling.

4.1 Phenological stages of wild species

The mean data on phenological stages of wild species viz., *P. guineense*, *P. chinensis*, *P. cattleianum* and *P. guajava* cv. Arka Kiran are presented in Table 1, Fig. 1-7 and Plate 8-11.

4.1.1 Time of leaf flushing

The days taken from bud bursting to leaf emergence ranged from six to nine in various species. It was high (9) in Arka Kiran followed by *P. cattleianum* (8) and *P. guineense* (7). It was found to be low (6) in *P. chinensis*.

4.1.2 Duration of flower bud initiation to full bloom

The days taken from flower bud initiation, (from when the flower bud becomes visible, to full bloom) ranged from 19 to 23 days. *P. guineense* recorded highest (23) number of days taken for full bloom followed by *P. chinensis* (21) and Arka Kiran (20). The lowest (19) number of days was taken by *P. cattleianum* to reach full bloom from flower bud initiation.

4.1.3 Time of flowering

The time of flowering ranged from February-April and August-September in *P. guineense*, *P. chinensis* and Arka Kiran whereas, it was June in *P. cattleianum*. The result indicated the synchronization of flowering between Arka Kiran and wild species *P. guineense* and *P. chinensis*.

4.1.4 Duration of flowering

The days taken from occurrence of first flowering to last flowering ranged from 30 to 65 days. The duration was highest (65) for Arka Kiran followed by *P. guineense* (60) and *P. chinensis* (58). It was observed that the lowest duration (30) was recorded for *P. cattleianum*.

4.1.5 Peak flowering

Days taken to 50 per cent flowering from the initial flowering were in the range between 15 to 30 days. Arka Kiran took longest time (30) to reach 50% flowering followed by *P. guineense* (28) and *P. chinensis* (22). Whereas, *P. cattleianum* took 15 days to reach peak flowering which was the lowest among all the other varieties/ species.

4.1.6 Anther dehiscence

The anther dehiscence, which was recorded and expressed in hours, observed in different species was in the range of 7.00 to 10.00 a.m. The *P. guineense* showed the earliest time (7.00 to 8.00 a.m) of anther dehiscence followed by *P. cattleianum* (7.30 to 8.30 a.m) and Arka Kiran (7.00 to 9.00 a.m). The

anther dehiscence was delayed in *P. chinensis* (8.00 to 10.00 a.m.) compared to other varieties/ species. Synchronization of anther dehiscence between wild species and guava cultivar was observed.

4.1.7 Pollen viability assessment

The per cent germination of pollen in the freshly opened flowers was in the range of 40.23 to 80.34. Arka Kiran showed the highest (80.34) pollen germination percent followed by *P. chinensis* (65.46) and *P. guineense* (62.30). The lowest (40.23) percent pollen germination was found to be in *P. cattleianum*.

4.1.8 Duration of flowering to fruit set

The days taken from anthesis to development of peanut stage fruit was in the range of 12 to 18 days. *P. guineense* took more (18) days from flowering to fruit set followed by *P. chinensis* (15) and Arka Kiran (15). *P. cattleianum* took less number of days (12) to attain fruit set when compared to other varieties/ species.

Table 1. Phenological stages of guava varieties and wild species

Varieties/ species	Time of leaf flushing (days)	Duration of flower bud initiation to full bloom (days)	Time of flowering (month)	Duration of flowering (days)	Peak flowering (days)	Anther dehiscence (time)	Pollen viability assessment (%)	Duration of flowering to fruit set (days)	Duration of fruit set to harvest (days)	Duration of fruiting (days)
<i>P. guineense</i>	7.00	23.00	Feb.- Apr. and Aug.-Sept.	60.00	28.00	7.00-8.00 a.m	62.30	18.00	103.0	80.00
<i>P.chinensis</i>	6.00	21.00	Feb-Apr and Aug.-Sept.	58.00	22.00	8.00-10.00 a.m	65.46	15.00	120.0	70.00
<i>P.cattleianum</i>	8.00	19.00	June	30.00	15.00	7.30-8.30 a.m	40.23	12.00	100.0	65.00
<i>P.guajava</i> var. Arka Kiran	9.00	20.00	Feb.-Apr. and Aug.-Sept.	65.00	30.00	7.00-9.00 a.m	80.34	15.00	120.0	120.0
Mean	7.50	20.75		49.50	23.75		62.08	15.00	110.75	78.75
SEm±	0.65	0.85		7.38	3.38		8.28	1.22	5.38	13.90
CV (%)	17.21	8.23		29.63	28.43		26.67	16.33	9.71	29.80

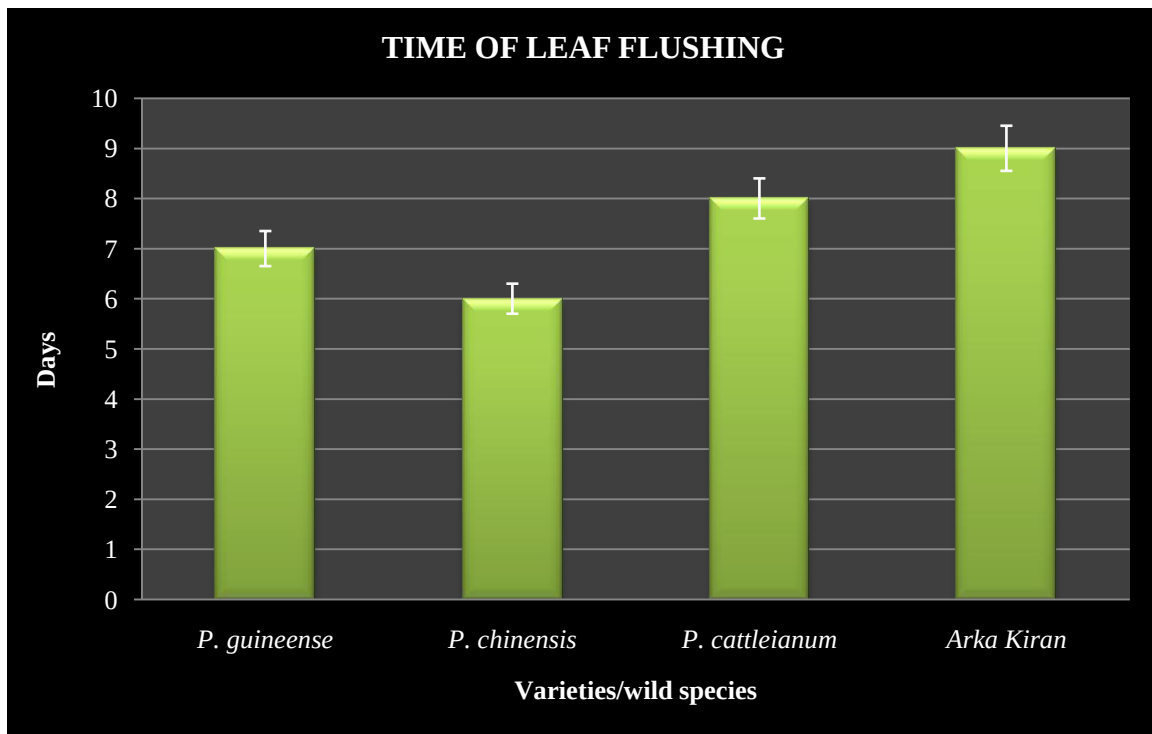


Fig. 1: Time of leaf flushing in guava varieties and wild species

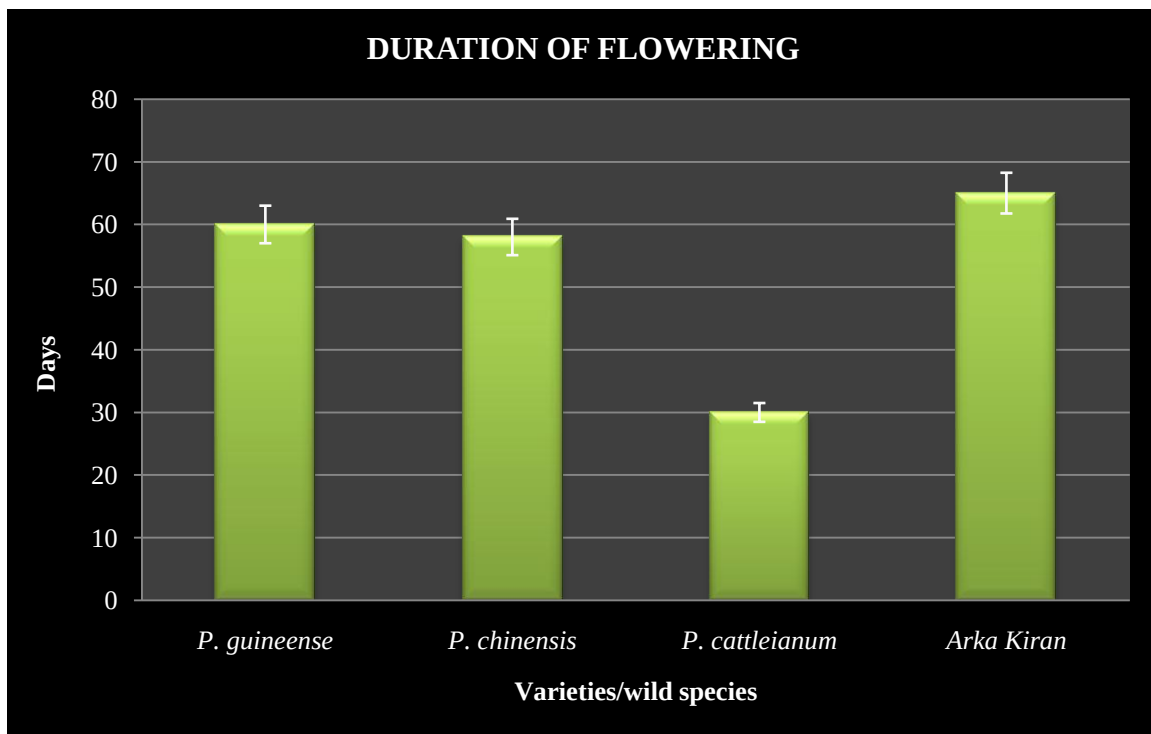


Fig. 2: Duration of flowering in guava varieties and wild species

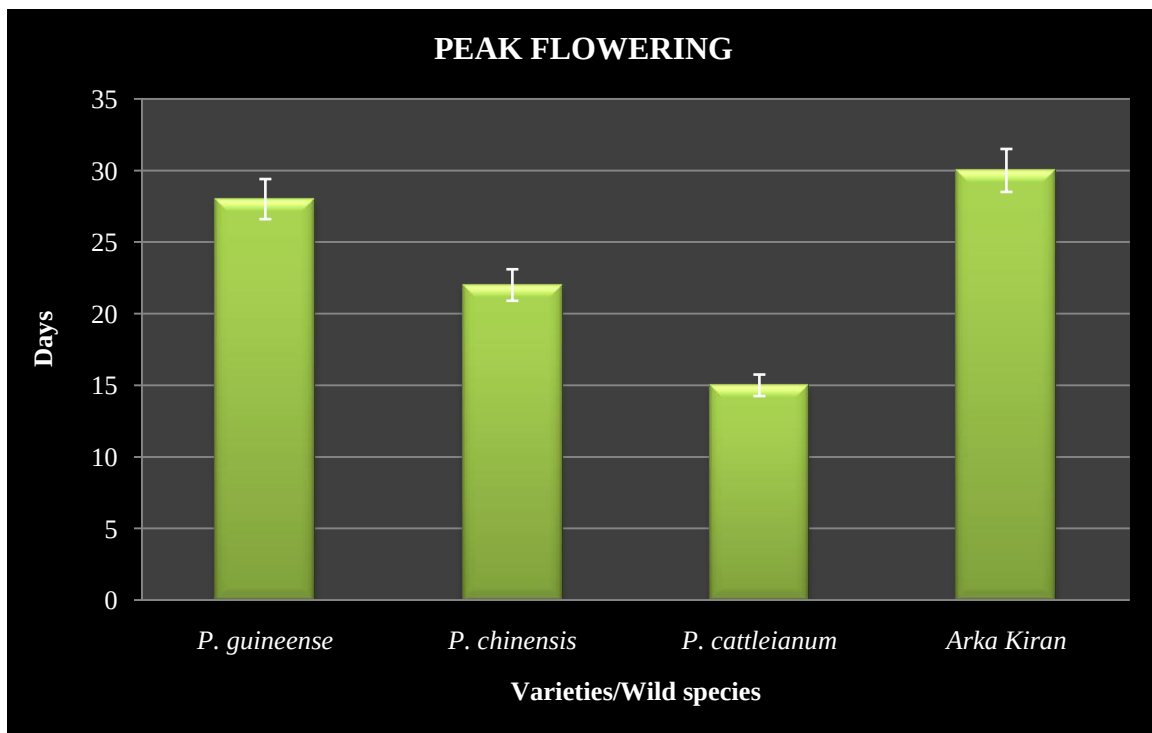


Fig. 3: Peak flowering in guava varieties and wild species

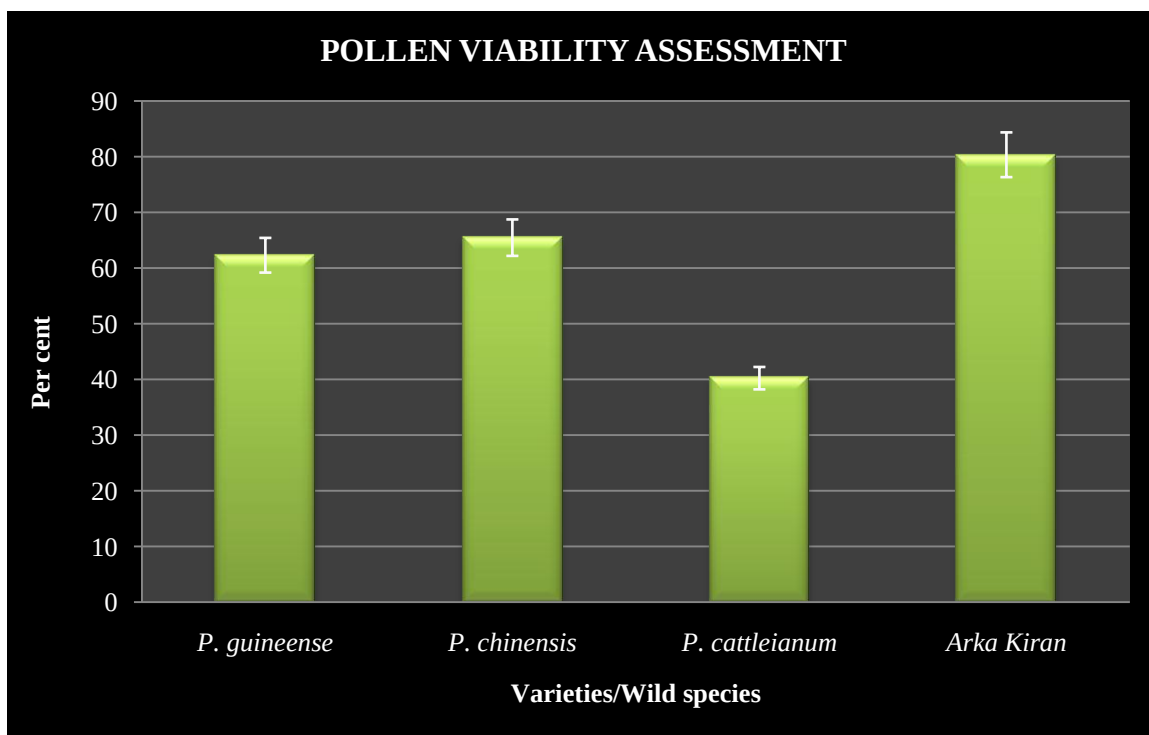


Fig. 4: Pollen viability assessment in guava varieties and wild species

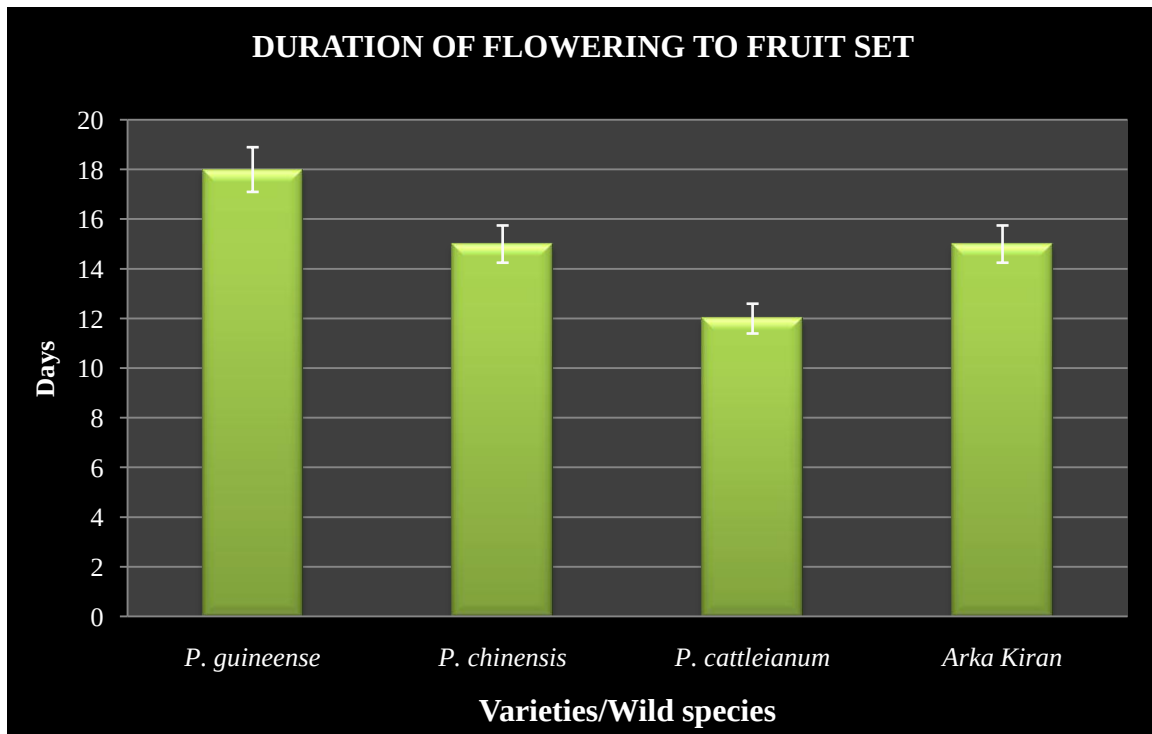


Fig. 5: Duration of flowering to fruit set in guava varieties and wild species

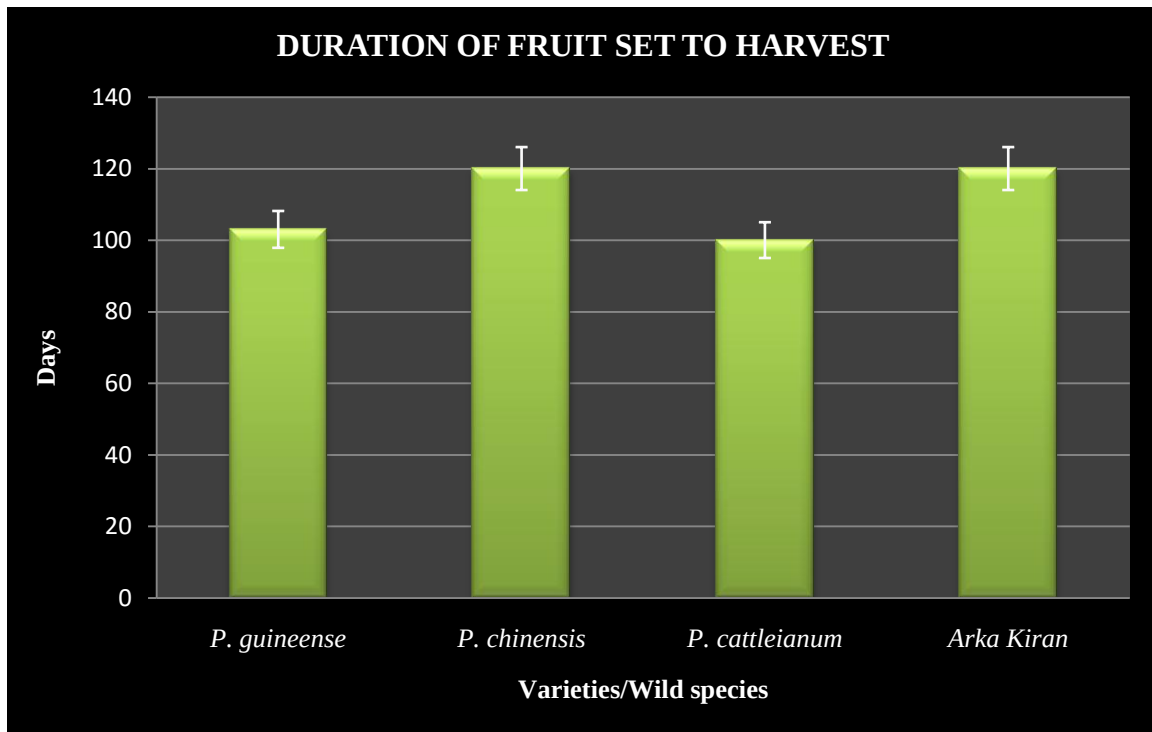


Fig. 6: Duration of fruit set to harvest in guava varieties and wild species

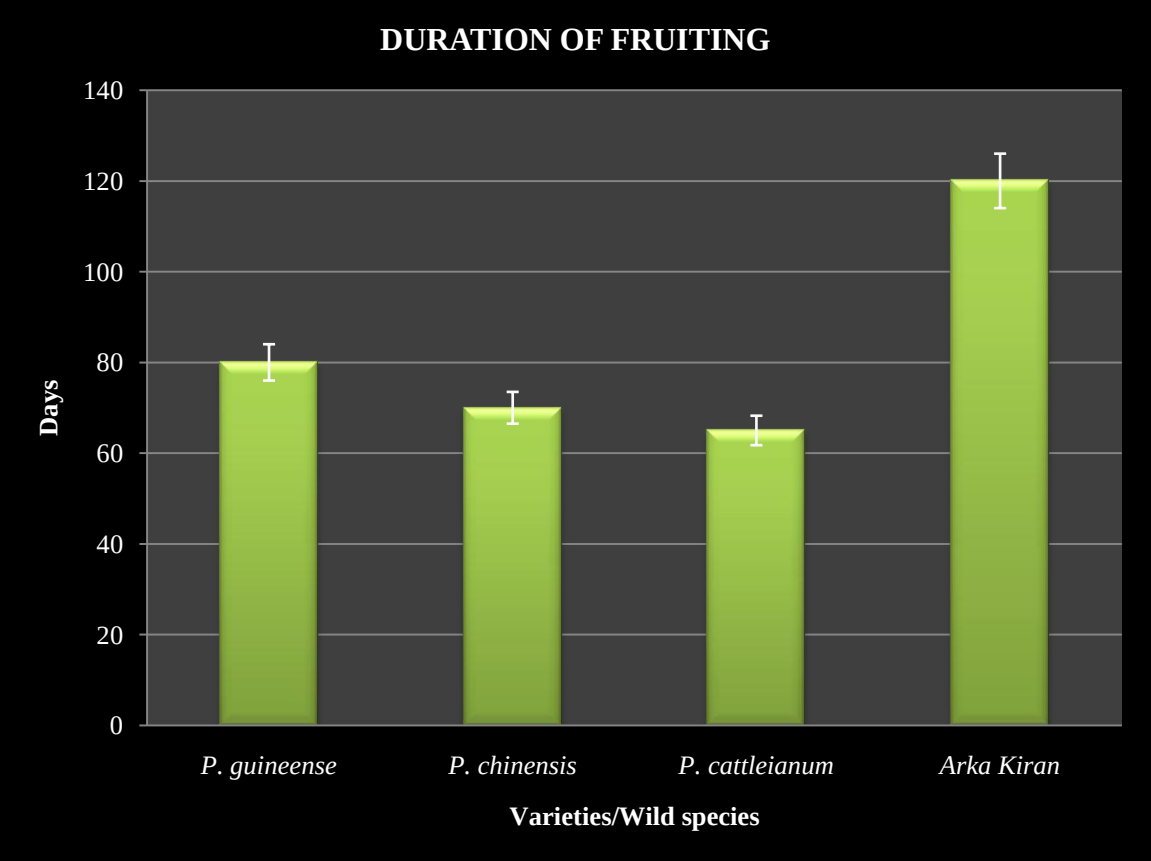


Fig. 7: Duration of fruiting in guava varieties and wild species

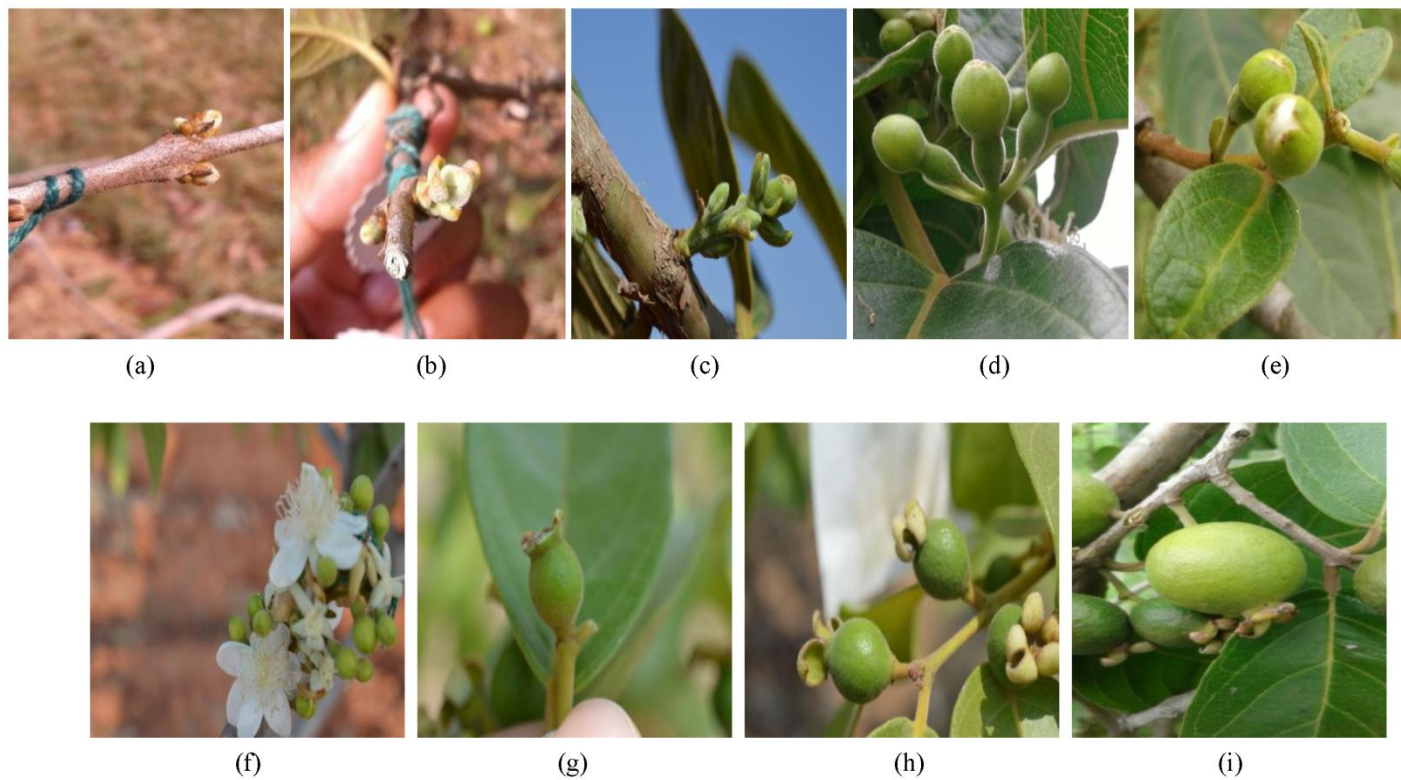


Plate 8. Phenological stages in *Psidium guineense*

(a) Bud differentiation, (b) Leaf emergence, (c) Flower bud initiation, (d) Flower bud development, (e) Flower bud ready to open, (f) Anthesis, (h) Fruit set, (i) Fruit development, (j) Ripening

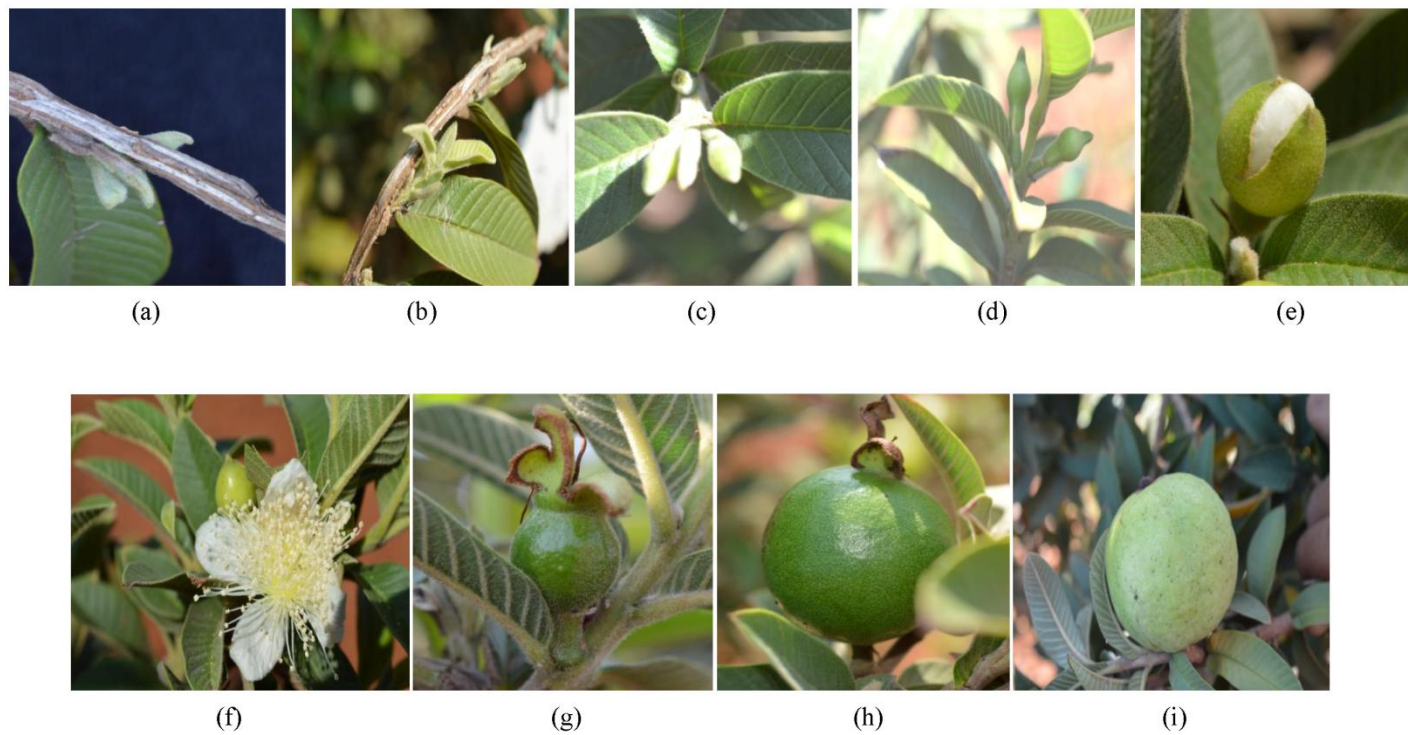


Plate 9. Phenological stages in *Psidium chinensis*

(a) Bud differentiation, (b) Leaf emergence, (c) Flower bud initiation, (d) Flower bud development, (e) Flower bud ready to open, (f) Anthesis, (g) Fruit set, (h) Fruit development, (i) Ripening

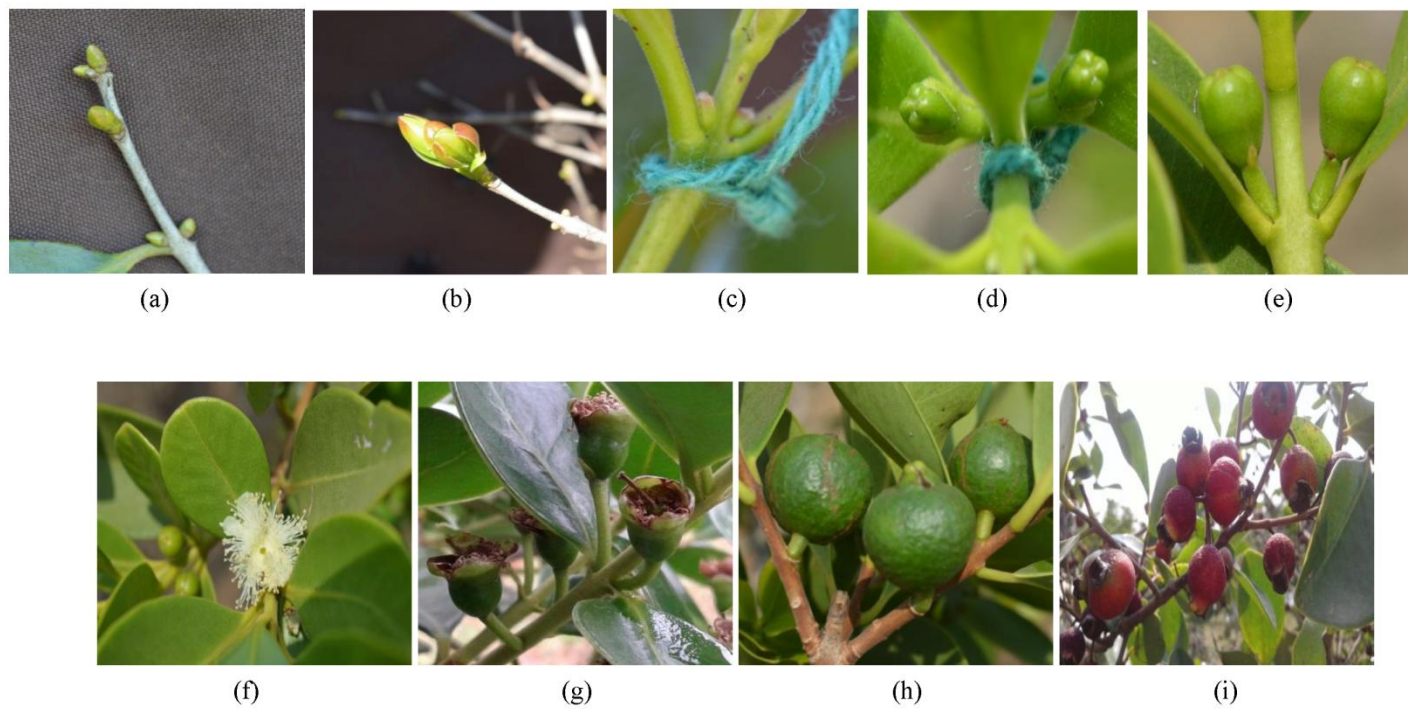
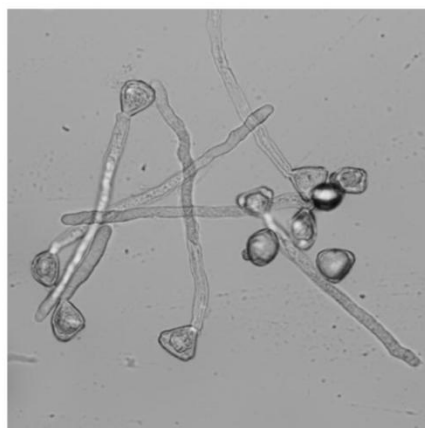
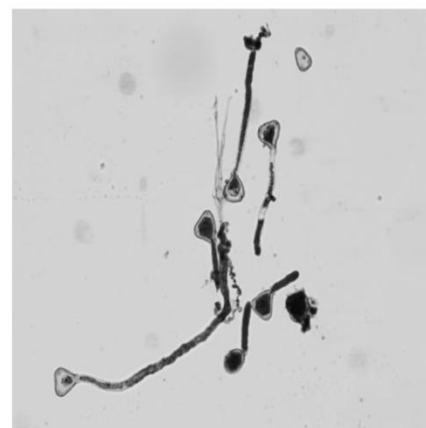


Plate 10. Phenological stages in *Psidium cattleianum*

(a) Bud differentiation, (b) Leaf emergence, (c) Flower bud initiation, (d) Flower bud development, (e) Flower bud ready to open, (f) Anthesis, (h) Fruit set, (i) Fruit development, (j) Ripening



(a) *Psidium guineense*



(b) *Psidium chinensis*



(c) *Psidium cattleianum*



(d) Arka Kiran

Plate 11. Pollen germination in guava varieties and wild species

4.1.9 Duration of fruit set to harvest

The days taken from fruit set (pea size) to horticultural maturity was in the range of 103 to 120 among the species and the variety Arka Kiran. The longest time (120) was recorded in Arka Kiran and *P. chinensis* followed by *P. guineense* (103) while the shortest period (100) was recorded in *P. cattleianum*.

4.1.10 Duration of fruiting

The days taken from first fruiting to last fruiting were in the range of 60 to 120. Arka Kiran showed the longest duration (120) of fruiting followed by *P. guineense* (80) and *P. chinensis* (70), whereas, *P. cattleianum* showed the shortest duration (65) of fruiting.

4.2 Interspecific hybridization using wild species

Previous experiments on the use of nutrient solution for preparing pollen slurry have resulted in improved fruit set. Hence, standardized nutrient solutions were used to enhance the pollen tube growth and the seed set. The comparative results of nutrient solution as against control are presented in Table 2 Fig. 8-10 and Plate 12.

4.2.1 Number of flowers crossed

The total number of flowers crossed using wild species under various treatments ranged from 11 to 100. The number of flowers crossed was high (100) in the treatment combination Arka Kiran $\times$ *P. chinensis* (Control) followed by Arka Kiran $\times$ *P. guineense* (Nutrient solution) with 62 flowers and Arka Rashmi $\times$ *P. guineense* (Nutrient solution) with 45 flowers. It was low (11) in the treatment combination Arka Rashmi $\times$ *P. chinensis* (Control) compared to other treatment combinations.

4.2.2 Number of fruits set

The total number of flowers crossed using wild species under various treatments ranged from 10 to 36. The number of flowers crossed was high (36) in the treatment combination Arka Rashmi $\times$ *P. chinensis* (Nutrient Solution) followed by Arka Kiran $\times$ *P. guineense* (control) with 32 flowers and Arka Kiran $\times$ *P. chinensis* (Nutrient solution) with 28 flowers. It was low (10) both in the treatment combination Arka Mridula $\times$ *P. guineense* (Nutrient Solution) and Arka Rashmi $\times$ *P. chinensis* (Control) compared to other treatment combinations.

4.2.3 Per cent fruit set

The per cent fruit set differed significantly and ranged from 32.00 to 97.07. The mean per cent fruit set was high (97.07) in the treatment combination Arka Rashmi $\times$ *P. chinensis* (Nutrient solution) followed by Arka Rashmi $\times$ *P. chinensis* (Control) with a set of 89.94 and Arka Mridula $\times$ *P. chinensis* (control) with a set of 80.67. The lowest (32.00) per cent fruit set was recorded in the treatment combination of Arka Kiran $\times$ *P. guineense* (Control).

4.2.4 Number of fruits harvested

The number of fruits harvested using wild species under various treatments differed significantly and ranged from 15 to 1. The number of fruits recovered was high (15) in the treatment combination Arka Kiran $\times$ *P. chinensis* (Nutrient solution) followed by Arka Kiran $\times$ *P. chinensis* (Control) with 9.67 fruits. The next best combination was Arka Rashmi $\times$ *P. chinensis* (Nutrient solution) which resulted in the recovery of 8.67 fruits.

The treatment combination Arka Rashmi $\times$ *P. guineense* (Control) and Arka Rashmi $\times$ *P. chinensis* (Control) resulted in the recovery of only one fruit.

4.2.5 Number of seeds obtained

The number of seeds harvested under various treatment combinations differed significantly. It was in the range of 6758.67 to 456.67. The highest (6758.67) number of seeds was obtained from the treatment combination of Arka Kiran $\times$ *P. chinensis* (Nutrient solution) followed by Arka Kiran $\times$ *P. chinensis* (Control) with a recovery of 5495 seeds and Arka Rashmi $\times$ *P. chinensis* (Nutrient solution) with 4211.67 seeds. The treatment combination of Arka Rashmi $\times$ *P. chinensis* (Control) resulted in lowest (456.67) seed recovery.

Table 2. Cross compatibility of guava varieties and wild species on fruit set and seed recovery

Treatments	Number of flowers crossed	Number of fruit set	Per cent fruit set	Number of fruits harvested	Per cent fruit harvested	Number of seeds obtained	Per cent germination
Arka Kiran $\times$ <i>P. guineense</i> (Control)	100.00	32.00	32.00 (34.45)	2.00	2.00 (8.13)	1223.00	90.00 (71.579)
Arka Kiran $\times$ <i>P. guineense</i> (Nutrient solution)	62.00	24.00	38.47 (38.47)	2.00	3.20 (10.30)	1672.67	98.21 (82.311)
Arka Mridula $\times$ <i>P. guineense</i> (Control)	38.00	21.00	50.00 (48.02)	1.33	2.60 (9.28)	850.00	80.42 (63.737)
Arka Mridula $\times$ <i>P. guineense</i> (Nutrient solution)	20.00	10.00	55.04 (45.00)	3.00	15.00 (22.78)	1658.33	84.00 (66.428)
Arka Rashmi $\times$ <i>P. guineense</i> (Control)	35.00	20.00	57.07 (49.11)	1.00	4.40 (9.80)	1464.33	92.85 (63.439)
Arka Rashmi $\times$ <i>P. guineense</i> (Nutrient solution)	45.00	17.00	38.00 (37.92)	3.00	2.90 (12.11)	2520.00	80.00 (74.491)
Arka Kiran $\times$ <i>P. chinensis</i> (Control)	25.00	19.00	75.94 (60.67)	9.67	40.30 (39.43)	5495.00	88.00 (69.743)
Arka Kiran $\times$ <i>P. chinensis</i> (Nutrient solution)	35.00	28.00	80.23 (63.44)	15.00	42.20 (40.53)	6758.67	94.00 (75.853)
Arka Mridula $\times$ <i>P. chinensis</i> (Control)	25.00	22.00	80.67 (69.74)	8.00	32.00 (34.45)	3342.00	2.80 (9.632)
Arka Mridula $\times$ <i>P. chinensis</i> (Nutrient solution)	20.00	16.00	88.62 (63.44)	8.00	40.00 (39.23)	3490.33	8.50 (16.950)
Arka Rashmi $\times$ <i>P. chinensis</i> (Control)	11.00	10.00	89.94 (72.44)	1.00	9.10 (17.56)	456.67	89.13 (70.750)
Arka Rashmi $\times$ <i>P. chinensis</i> (Nutrient solution)	37.00	36.00	97.07 (80.53)	8.67	24.10 (29.40)	4211.67	99.0 (84.261)
CV (%)	2.38	4.85	0.92	13.19	1.95	2.63	0.78
SEm$\pm$	0.30	0.34	0.17	0.23	0.15	24.19	0.16
CD (P=0.05)	0.88	1.01	0.87	0.67	0.75	70.93	0.82

* Figures given in parantheses indicate arc sin values

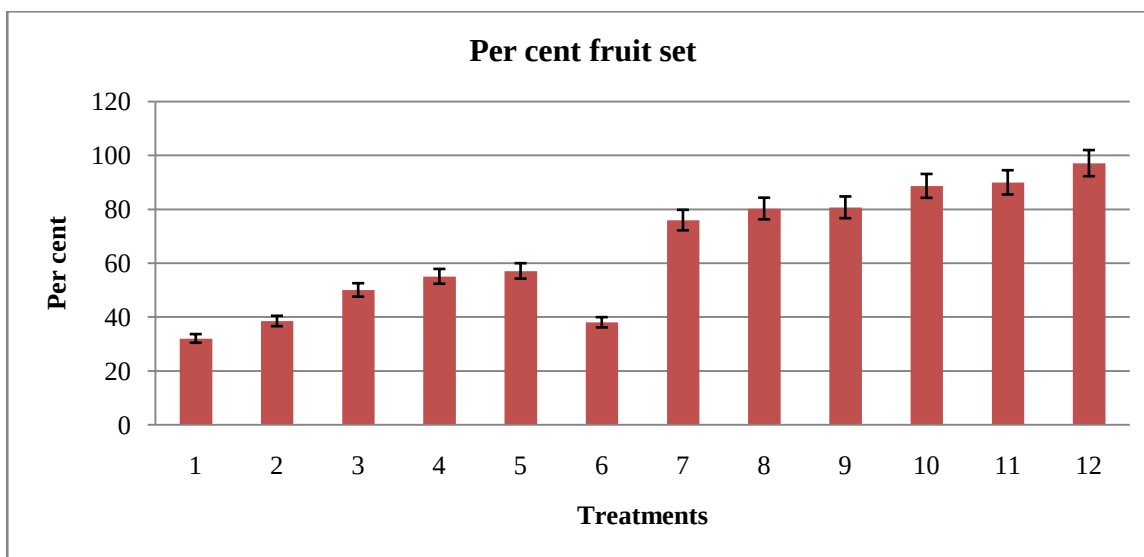


Fig. 8: Per cent of fruit set in different cross combinations

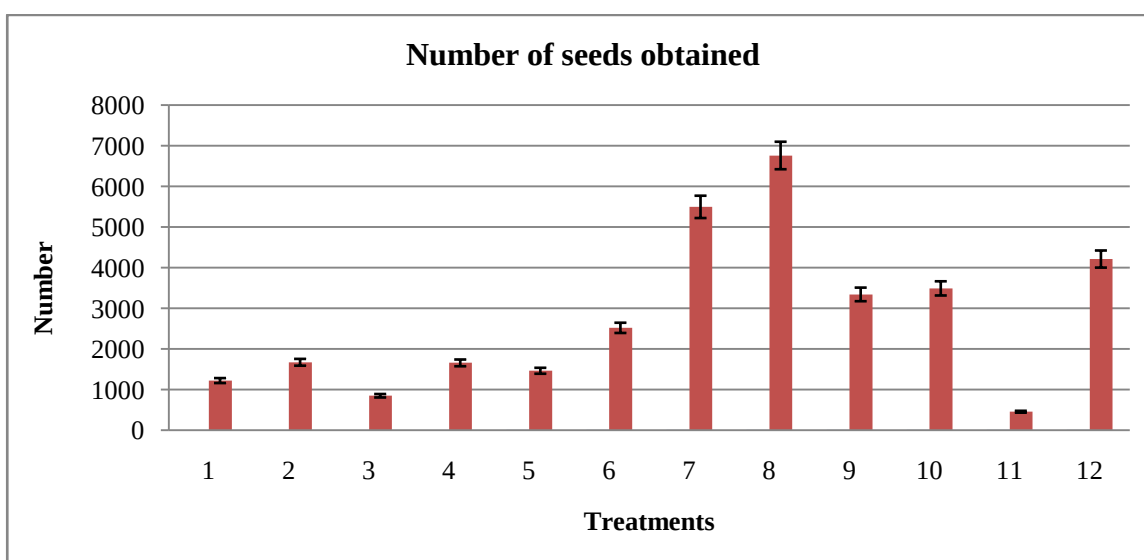


Fig. 9: Number of seeds obtained in different cross combinations

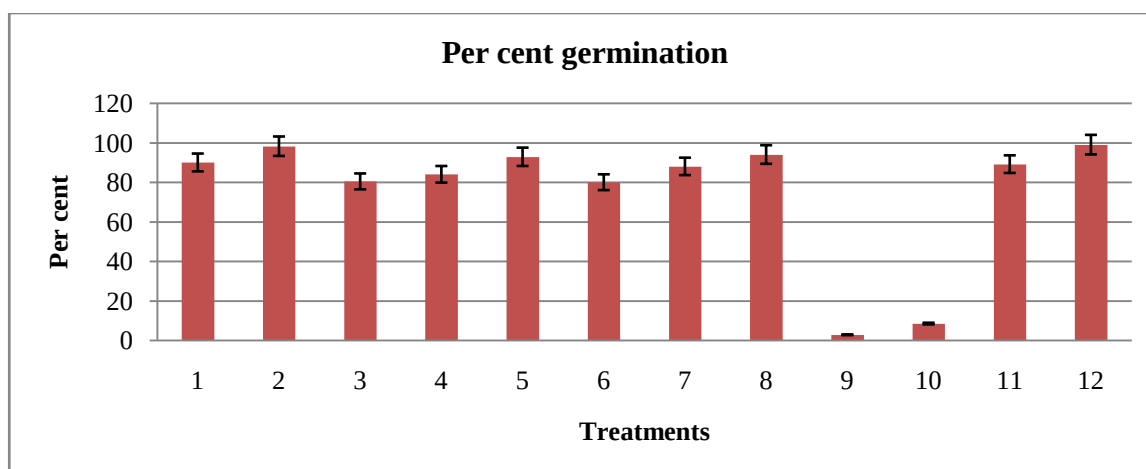


Fig. 10: Per cent germination of seeds obtained from different cross combinations

1: Arka Kiran $\times$ *P. guineense* (Control), **2:** Arka Kiran $\times$ *P. guineense* (Nutrient solution), **3:** Arka Mridula $\times$ *P. guineense* (Control), **4:** Arka Mridula $\times$ *P. guineense* (Nutrient solution), **5:** Arka Rashmi $\times$ *P. guineense* (Control), **6:** Arka Rashmi $\times$ *P. guineense* (Nutrient solution), **7:** Arka Kiran $\times$ *P. chinensis* (Control), **8:** Arka Kiran $\times$ *P. chinensis* (Nutrient solution), **9:** Arka Mridula $\times$ *P. chinensis* (Control), **10:** Arka Mridula $\times$ *P. chinensis* (Nutrient solution), **11:** Arka Rashmi $\times$ *P. chinensis* (Control), **12:** Arka Rashmi $\times$ *P. chinensis* (Nutrient solution)

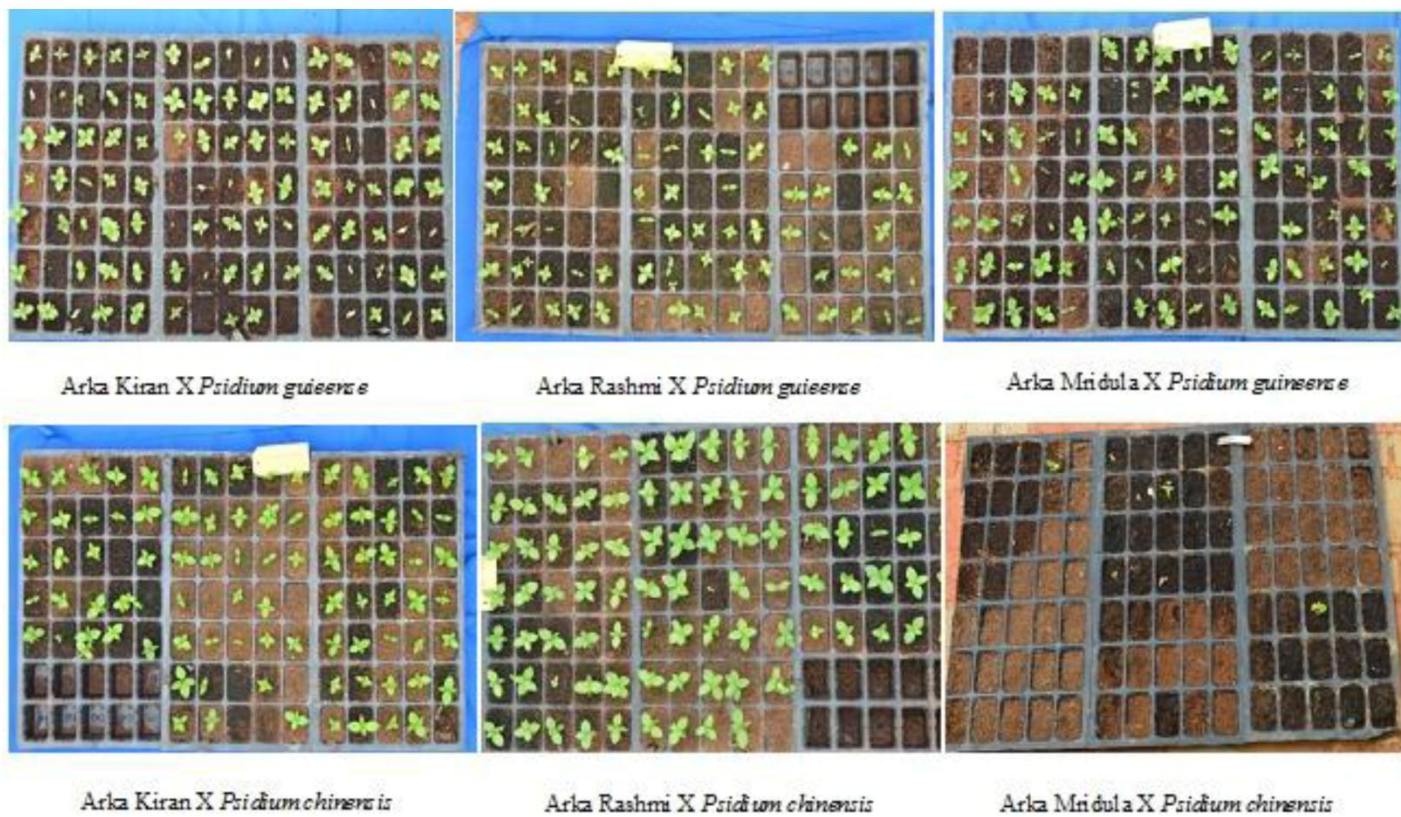


Plate 12. Seedling emergence of interspecific hybrids

4.2.6 Per cent germination

Seeds from each treatment combination were sown and germination percent obtained was in the range of 2.8 to 99. The highest (99) per cent germination was obtained from the treatment combination of Arka Rashmi × *P. chinensis* (Nutrient solution) followed by Arka Kiran × *P. guineense* (Nutrient solution) with a germination per cent of 98.21 and Arka Rashmi × *P. guineense* (Control) with 92.85 per cent. The treatment combination of Arka Mridula × *P. chinensis* (Control) resulted in lowest (2.8) per cent germination.

4.3 Cytological studies of different varieties and wild species

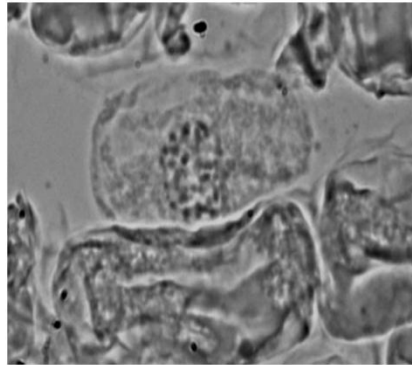
Cytological study was carried out to count the chromosome number or ploidy using root tips obtained from young sprouts of *P. guajava* cvs (Arka Amulya, Arka Kiran, Arka Mridula, Arka Rashmi, Local Red, Purple guava, Seedless triploid and Thailand guava) and the wild species (*P. cattelianum*, *P. chinensis* and *P. guineense*) as the interspecific hybridization would bring in sterility due to incompatibility.

The observation showed that the chromosome obtained were not in active mitotic metaphase (Plate 13.) to count the exact number $2n=22$ in *Psidium guajava* varieties, $2n = 77$ in *P. cattelianum* and $2n = 44$ in *P. guineense*. Hence, further standardization on best time of sample collection, stage and types of sample collection (shoot tip) is required to obtain the peak mitotic metaphase for counting the exact number.

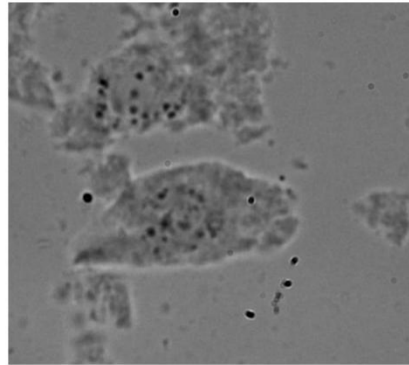
4.4 Morphological traits of guava varieties and wild species

4.4.1 Fruit traits

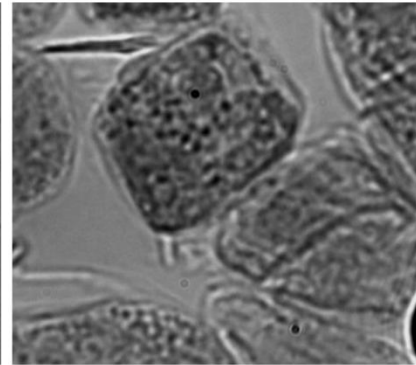
The fruit traits (Fruit weight (g), Fruit volume (ml), Fruit length (cm), Fruit width (cm), Thickness of outer pulp in relation to core diameter (cm), Fruit shape and Pericarp/peel colour of different guava varieties and the wild species are presented in Table 3, Fig. 11-12 and Plate 14.



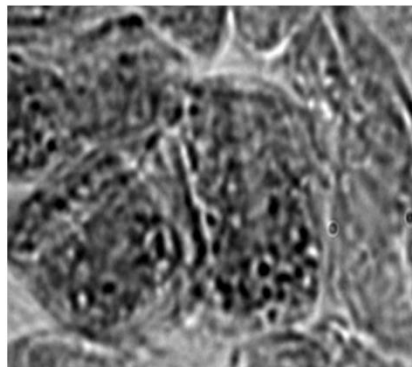
Arka Amulya



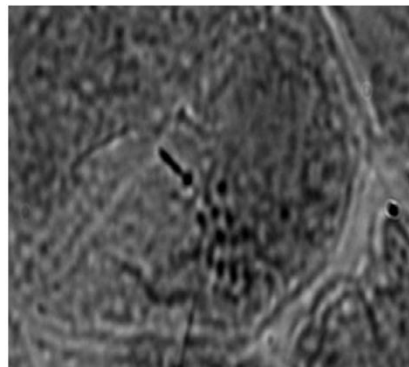
Thailand Guava



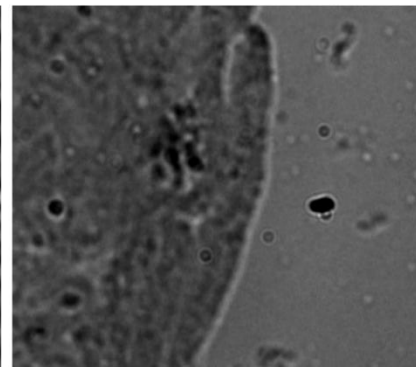
Arka Rashmi



Arka Kiran



Psidium guineense



Psidium chinensis

Plate 13. Cytological studies in guava varieties and wild species

4.4.1.1 Fruit weight

The fruit weight of different varieties and wild species of guava differed significantly and ranged from 22.90 to 196.67 cm, among which Allahabad Safeda was showing the highest (196.67cm) fruit weight followed by Arka Rashmi (173.53 cm) and Arka Amulya (173.29 cm) whereas, the lowest (22.90 cm) value was given by *P. cattleianum*.

4.4.1.2 Fruit volume

The fruit volume of different varieties and wild species of guava showed significant difference and ranged from 15.2 to 170 ml, among which Allahabad Safeda registered the highest (170.00 ml) followed by Arka Rashmi (160.00 ml) and Arka Amulya (153.70 ml) whereas, the lowest (15.2 ml) value was recorded in *P. cattleianum*.

4.4.1.3 Fruit length

The fruit length of different varieties and wild species of guava registered significant difference and it ranged from 2.90 to 5.93 cm. The highest (5.93 cm) fruit length was recorded by Allahabad Safeda followed by Local red (5.33 cm) and Arka Mridula (5.23 cm). The lowest value (2.90 cm) was registered by *P. cattleianum*.

4.4.1.4 Fruit width

The fruit width of different varieties and wild species of guava recorded significant difference and it ranged from 3.00 to 5.80 cm. Similar to fruit length and volume, Allahabad Safeda showed the highest (5.80 cm) followed by Arka Mridula (5.37 cm) and Arka Amulya (5.10 cm) whereas, the lowest (3.00 cm) was recorded in *P. cattleianum*.

4.4.1.5 Thickness of outer pulp in relation to core diameter

The thickness of outer pulp in relation to core diameter of different varieties and wild species of guava showed significant difference and it ranged from 1.31 cm to 0.45 cm, among which Arka Mridula registered the highest value (1.31 cm) followed by Arka Rashmi (1.23 cm) and Arka Amulya (1.20 cm) whereas, the lowest value (0.45 cm) was given by *P. guineense*.

Table 3. Fruit morphological traits of guava varieties and wild species.

Treatments	Fruit weight (g)	Fruit volume (ml)	Fruit length (cm)	Fruit width (cm)	Thickness of outer pulp in relation to core diameter (cm)	Fruit shape	Pericarp/ peel colour
Allahabad Safeda	196.67	170.00	5.93	5.80	1.10	Globose	Yellow white
ArkaAmulya	173.29	153.70	4.87	5.10	1.20	Globose	Grey yellow
ArkaKiran	153.53	145.00	5.00	4.93	1.10	Globose	Yellow white
ArkaMridula	147.67	134.70	5.23	5.37	1.31	Globose	Yellow white
ArkaRashmi	173.53	160.00	5.20	5.07	1.23	Globose	Grey yellow
Local Red	94.33	94.30	5.33	4.50	0.57	Sub-globose	Grey yellow
<i>P.cattleianum</i>	22.90	15.20	2.90	3.00	0.47	Globose	Red
<i>P. chinensis</i>	29.70	29.20	3.17	3.20	0.63	Globose	Yellow white
<i>P. guineense</i>	31.41	23.00	3.40	3.23	0.45	Sub-globose	Grey yellow
Purple Guava	64.50	52.70	4.50	4.43	0.60	Globose	Purple
Seedless Triploid	86.42	85.90	4.23	3.93	0.89	Oblong	Yellow white
Mean	106.72	96.70	4.52	4.41	0.87		
SEm±	4.45	4.80	0.13	0.12	0.05		
CV (%)	17.70	21.30	12.08	11.14	34.78		
CD(P=0.05)	13.06	14.00	0.38	0.35	0.14		

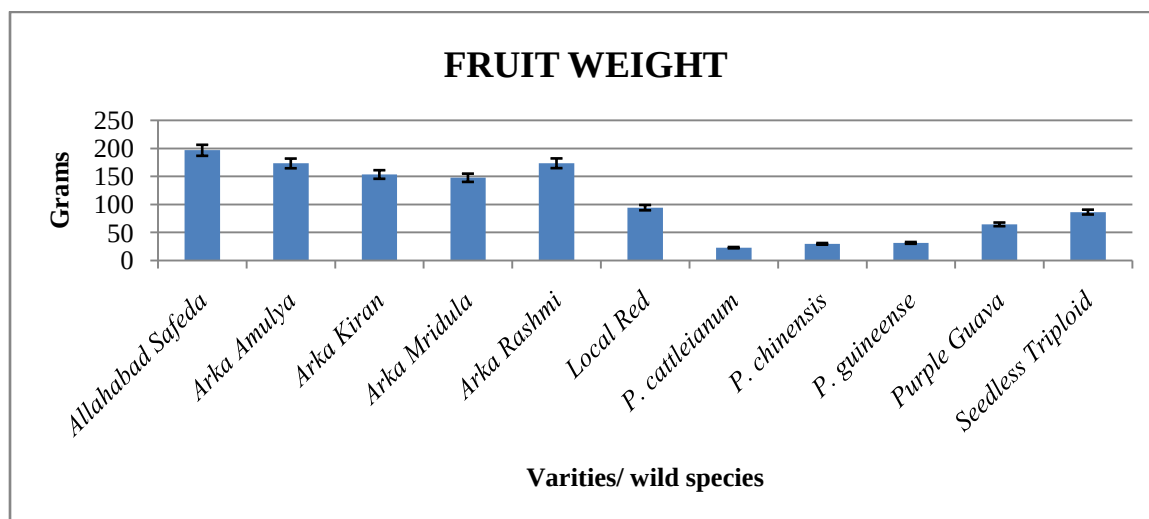


Fig. 11: Fruit weight of guava varieties and wild species

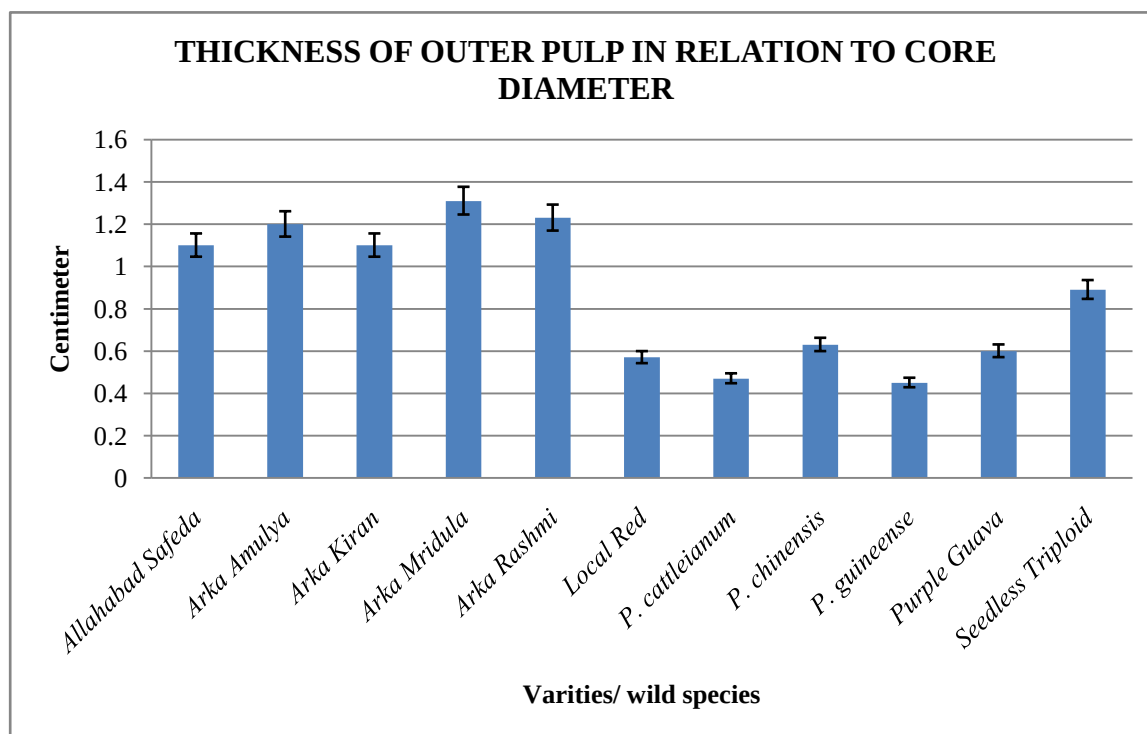


Fig. 12: Thickness of outer pulp in relation to core diameter of guava varieties and wild species

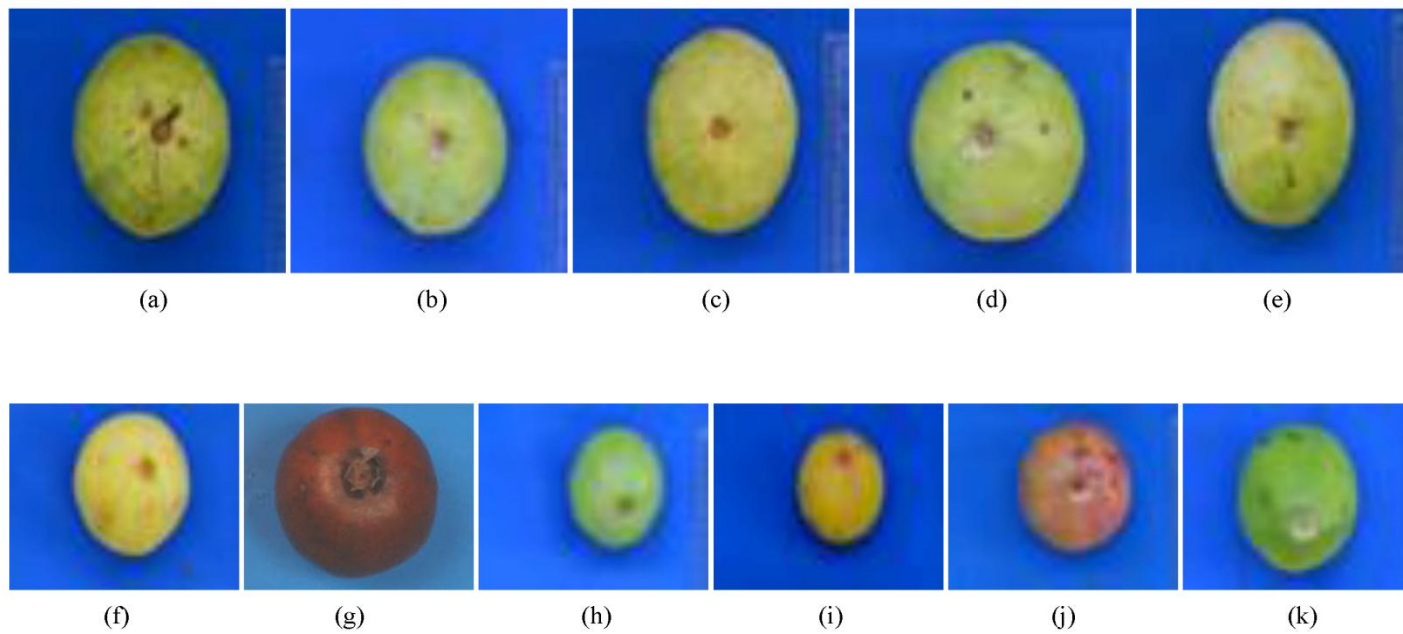


Plate 14. Fruit traits of guava varieties and wild species

(a) Allahabad Safeda, (b) Arka Amulya, (c) Arka Kiran, (d) Arka Mridula, (e) Arka Rashmi, (f) Local Red
 (g) *P. cattleianum*, (h) *P. chinensis*, (i) *P. guineense*, (j) Purple Guava, (k) Seedless Triploid

4.4.1.6 Fruit shape

The fruit shape was round or globose for all varieties and wild species except for Local Red and *P. guineense* which were sub-globose. The fruit shape was oblong in Seedless Triploid

4.4.1.7 Pericarp/ peel colour

The peel colour of Allahabad safeda, Arka Amulya, Arka Mridula, *P. chinensis* and Seedless Triploid were recorded to be yellow white whereas, that of Arka Kiran, Arka Rashmi, Local Red and *P. guineense* were recorded as grey yellow. The peel colour of *P. cattleianum* was found to be red and that of Purple guava was found to be purple.

4.4.2 Pulp and Seed traits

The pulp and seed traits (Core diameter (cm), 100 seed weight (g), Seed number, Seed hardness (Kg cm^{-2}) and pulp colour) of different guava varieties and the wild species are presented in Table 4, Fig. 13 and Plate 15.

4.4.2.1 Core diameter

The fruit core diameter of different varieties and wild species of guava registered significant difference among the varieties and it ranged from 2.80 to 4.80 cm. Arka Rashmi registered the highest (4.80 cm) fruit core diameter followed by Arka Mridula (4.70 cm) and Arka Amulya (4.67 cm) whereas, the lowest value (2.80 cm) was registered by *P. cattleianum*.

4.4.2.2 Seed weight

The 100 seed weight of different varieties and wild species of guava recorded significant difference and it ranged from 0.06 to Arka Amulya (0.77 g) and *P. chinensis* (0.75 g) whereas, the lowest value (0.06 g) was given by Seedless Triploid.

Table 4: Pulp and seed traits of guava varieties and wild species

Treatments	Core diameter (cm)	100 seed weight (g)	Seed number	Seed hardness (kg cm⁻²)	Pulp colour
Allahabad Safeda	4.47	1.07	284.67	10.70	White
ArkaAmulya	4.53	0.77	290.33	9.67	White
ArkaKiran	4.67	0.72	324.33	11.50	Red
ArkaMridula	4.70	0.72	317.33	9.33	White
ArkaRashmi	4.80	0.74	363.00	11.83	Red
Local Red	4.50	0.60	278.67	10.83	Red
<i>P.cattleianum</i>	2.80	0.39	80.33	11.17	Grey orange
<i>P. chinensis</i>	3.20	0.75	179.00	5.00	Red
<i>P. guineense</i>	3.00	0.67	187.00	13.33	Yellow white
Purple Guava	4.00	0.73	290.00	6.33	Red purple
Seedless Triploid	4.00	0.06	19.00	10.00	White
Mean	4.06	0.66	237.61	9.97	
SEm±	0.14	0.02	11.21	0.37	
CV (%)	15.01	14.22	20.01	15.86	
CD (P= 0.05)	0.41	0.06	32.87	1.09	

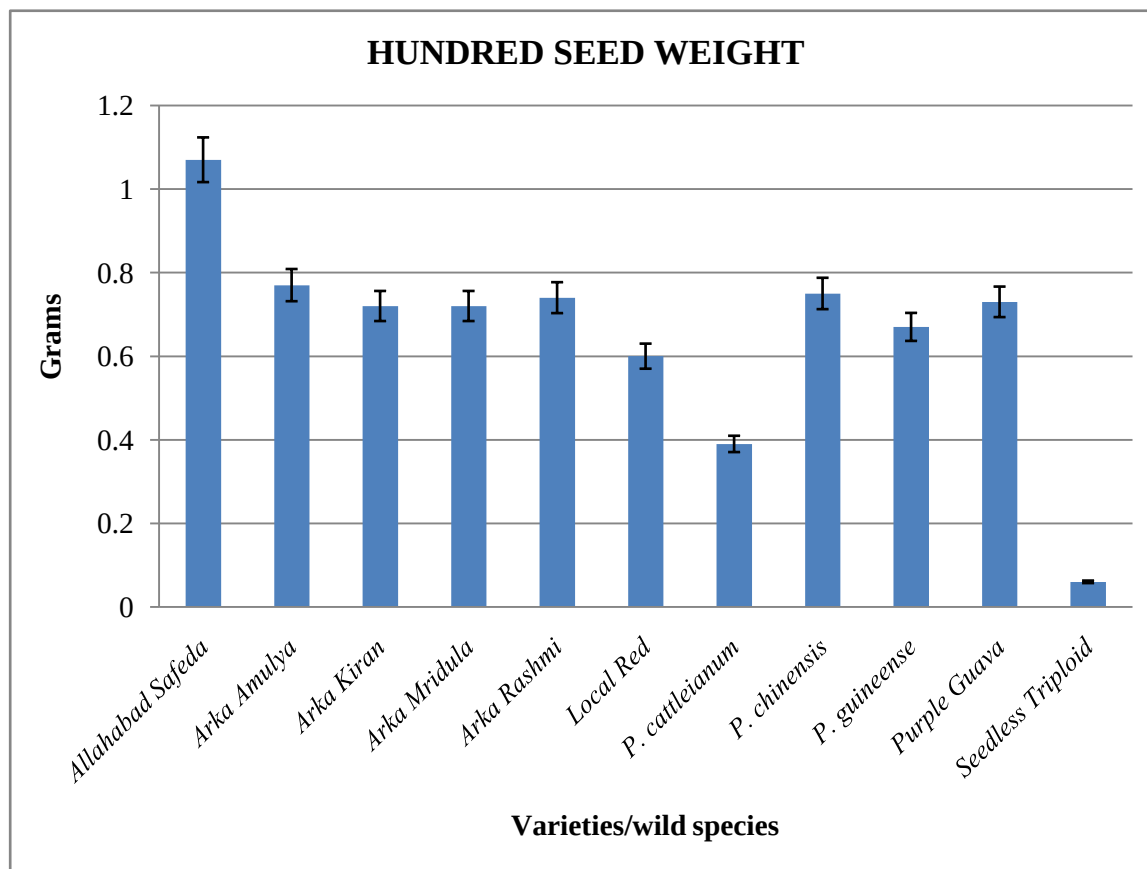


Fig. 13: Hundred seed weight of guava varieties and wild species

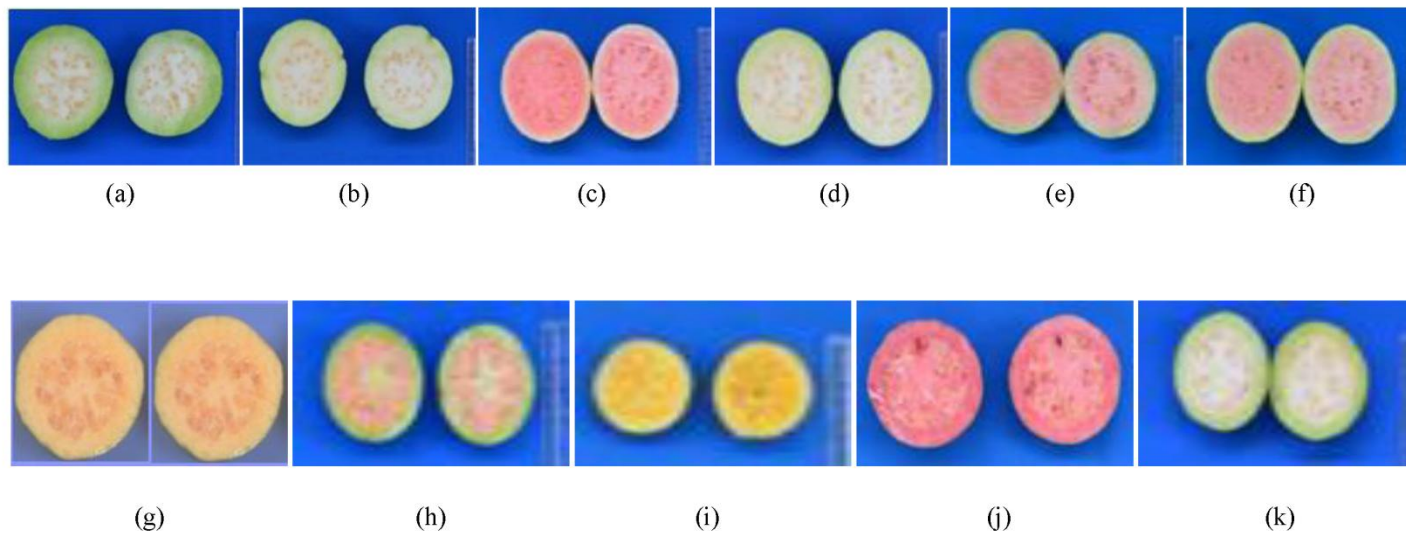


Plate 15. Fruit pulp and seed traits of guava varieties and wild species

(a) Allahabad Safeda, (b) Arka Amulya, (c) Arka Kiran, (d) Arka Mridula, (e) Arka Rashmi, (f) Local Red, (g) *P. cattleianum*, (h) *P. chinensis*, (i) *P. guineense*, (j) Purple Guava, (k) Seedless Triploid

4.4.2.3 Seed number

The seed number of different varieties and wild species of guava exhibited significant difference and it ranged from 19 to 363, among which Arka Rashmi registered the highest seed weight (363) followed by Arka Kiran (324.33) and Arka Mridula (317.33) whereas, the lowest value (19) was given by Seedless Triploid.

4.4.2.4 Seed hardness

The seed hardness of different varieties and wild species of guava showed significant difference and it ranged from 5 to 13.33 kg cm⁻², among which *P. guineense* registered the highest value (13.33) followed by Arka Rashmi (11.83) and Arka Kiran (11.50) whereas, the lowest value (5) was given by *P. chinensis*.

4.4.2.5 Pulp colour

The pulp colour of guava varieties viz; Arka Kiran, Arka Rashmi, Local Red and *P. chinensis* was found to be red, whereas it was found to be grey orange in *P. cattleianum*. In the species *P. guineense* it was found to be yellow white and in Purple Guava it was found to be red purple. All other varieties and wild species of guava showed white pulp colour.

4.5 Biochemical analysis

The biochemical traits (TSS (⁰B), Titrable Acidity (%), Vitamin C (mg 100 g⁻¹), Total sugar (g), Reducing sugar (g) and Non-reducing sugar (g)) of different varieties and wild species of guava are presented in Table 5, Fig. 14-18.

4.5.1 Total soluble solids

Among the different varieties and wild species of guava recorded significant difference 7.73 to 11.87⁰B. Arka Amulya and Arka Mridula showed the highest TSS (11.87⁰B) followed by Purple Local (11.73⁰B), whereas the lowest value (7.73⁰B) was shown by *P. cattleianum*.

4.5.2 Titrable Acidity

Among the different varieties and wild species of guava, in rind *P. cattleianum* registered significantly highest value (1.28 %) followed by Local Red (0.81 %) and Purple Guava (0.78 %), whereas, the lowest value was shown by *P. guineense* (0.59 %). In pulp, *P. cattleianum* registered highest titrable acidity (0.87 %) followed by Arka Rashmi (0.62 %) and Purple Guava (0.56 %), whereas the lowest value (0.37 %) was recorded by Seedless Triploid.

4.5.3 Vitamin C

The vitamin C content of different varieties and wild species of guava exhibited significant difference. Among the different varieties and wild species of guava, in rind Arka Rashmi showed highest ascorbic acid (260.43 mg 100g⁻¹ FW) followed by Arka Kiran (256.50 mg 100g⁻¹ FW) and *P. cattleianum* (242.53 mg 100g⁻¹ FW), whereas the lowest value (77.27 mg 100g⁻¹ FW) showed by *P. guineense*. In pulp, Arka Rashmi showed highest ascorbic acid (246.60 mg 100g⁻¹ FW) followed by Arka Kiran (228.32 mg 100g⁻¹ FW) *P. cattleianum* (210.26 mg 100g⁻¹ FW), whereas the lowest value (55.96 mg 100g⁻¹ FW) showed by *P. guineense*.

4.5.4 Total sugars

Among the different varieties and wild species of guava showed significant difference. In rind T₈- *P. chinensis* showed significantly the highest total sugar (7.52%) followed by Seedless triploid (7.17%) and Purple guava (7.10%), whereas the lowest value (2.65%) was shown by *P. cattleianum*. In pulp, Arka Kiran showed the highest total sugar (9.13 %) followed by Purple Guava (9.04 %) and Arka Rashmi (8.77 %), whereas the lowest value was shown by Local Red (2.66%).

4.5.5 Reducing sugars

Among the different varieties and wild species of guava, reducing sugar content exhibited significant difference. In rind Seedless Triploid showed highest reducing sugar (5.29 %) followed by *P. guineense* (5.26 %) and Purple Guava (4.85 %), whereas the lowest value (1.32 %) was recorded in *P. cattleianum*. In pulp, Arka Rashmi showed highest reducing sugar (7.26 %) followed by Arka Kiran (7.00 %) and Allahabad Safeda (5.42 %), whereas the lowest value (3.64 %) recorded in Arka Amulya.

Table 5: Biochemical traits of guava varieties and wild species

Treatments	TSS (°B)	Titrable acidity (%)		Vitamin C (mg 100g ⁻¹)		Total sugars (g)		Reducing sugar (g)		Non reducing sugar (g)	
		Rind	Pulp	Rind	Pulp	Rind	Pulp	Rind	Pulp	Rind	Pulp
Allahabad Safeda	10.80	0.60	0.36	180.13	152.01	7.07	8.37	4.30	5.42	2.52	2.60
Arka Amulya	11.87	0.67	0.47	153.10	125.04	6.12	6.86	3.61	3.64	3.11	3.15
Arka Kiran	10.80	0.66	0.41	256.50	228.32	4.30	9.13	1.89	7.00	1.27	1.63
Arka Mridula	11.87	0.62	0.47	143.13	122.94	6.48	7.13	3.75	3.65	3.06	3.11
Arka Rashmi	10.67	0.71	0.62	260.43	246.60	3.11	8.77	1.88	7.26	1.53	1.44
Local Red	9.93	0.81	0.47	122.60	108.07	2.86	2.66	1.40	1.71	1.19	1.23
<i>P. cattleianum</i>	7.73	1.28	0.87	242.53	210.26	2.65	5.49	1.32	4.45	1.26	1.48
<i>P. chinensis</i>	9.67	0.69	0.55	232.47	165.88	7.52	8.56	4.47	5.13	2.66	3.13
<i>P. guineense</i>	11.13	0.60	0.46	77.27	55.96	6.38	7.67	5.26	4.53	1.03	3.52
Purple Guava	11.73	0.78	0.56	91.67	82.78	7.10	9.04	4.85	4.48	2.57	4.56
Seedless Triploid	11.53	0.68	0.37	124.10	95.57	7.17	8.49	5.29	5.21	1.88	3.20
Mean	10.70	0.74	0.51	171.27	144.86	5.52	7.47	3.46	4.77	1.84	2.64
SEM±	0.29	0.01	0.01	1.07	0.44	0.09	0.08	0.06	0.09	0.05	0.07
CV (%)	11.60	4.09	5.72	2.64	1.29	7.57	5.27	7.05	8.84	10.99	11.16
CD (P=0.05)	0.86	0.02	0.02	3.13	1.29	0.28	0.25	0.16	0.25	0.13	0.20

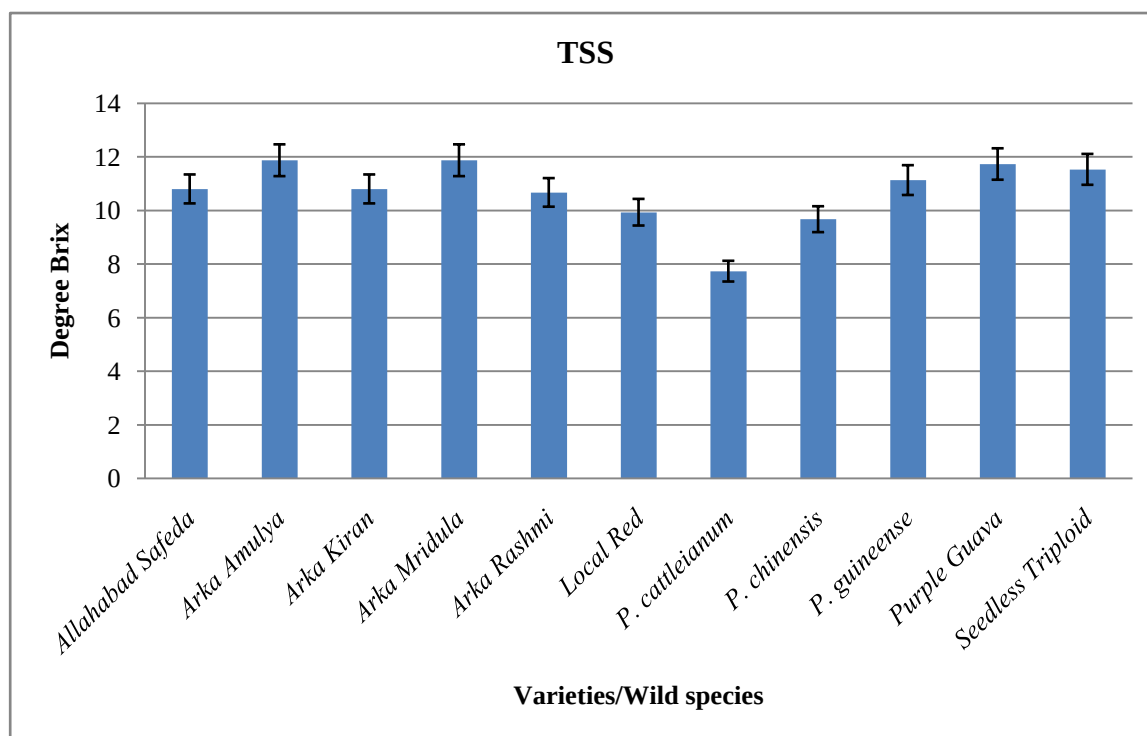


Fig. 14: TSS of guava varieties and wild species

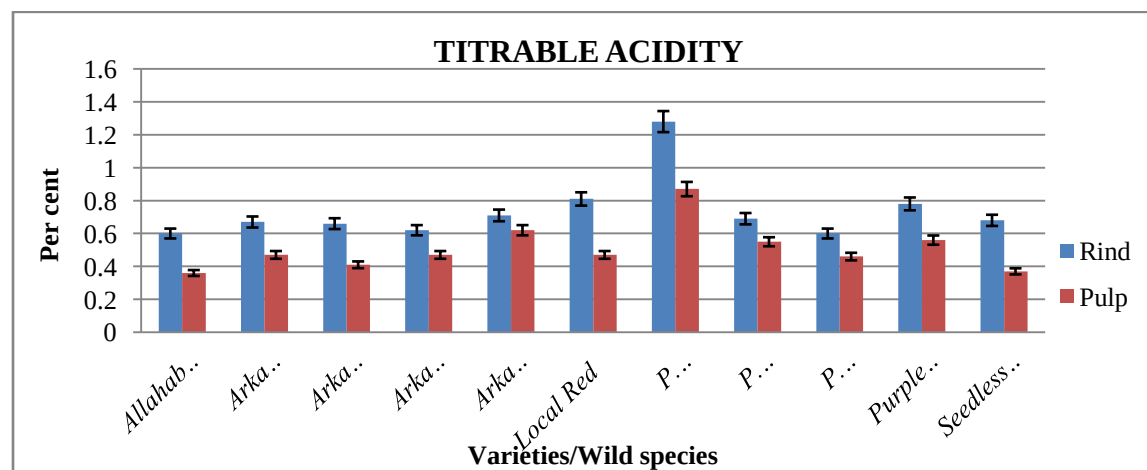


Fig. 15: Titrable acidity of guava varieties and wild species

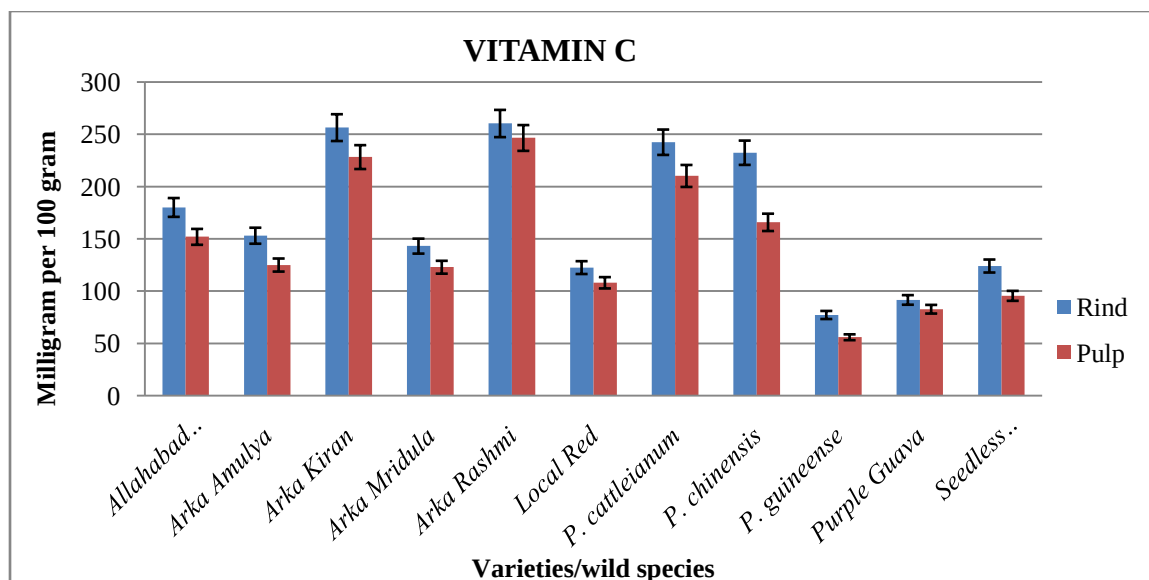


Fig. 16: Vitamin C content of guava varieties and wild species

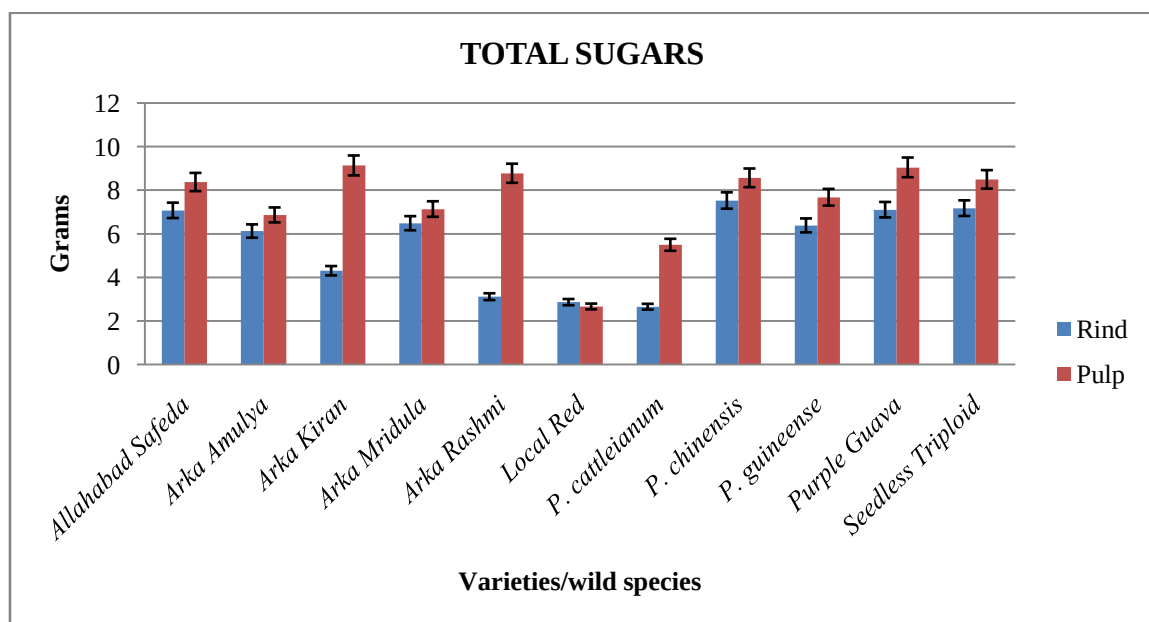


Fig. 17: Total sugars of guava varieties and wild species

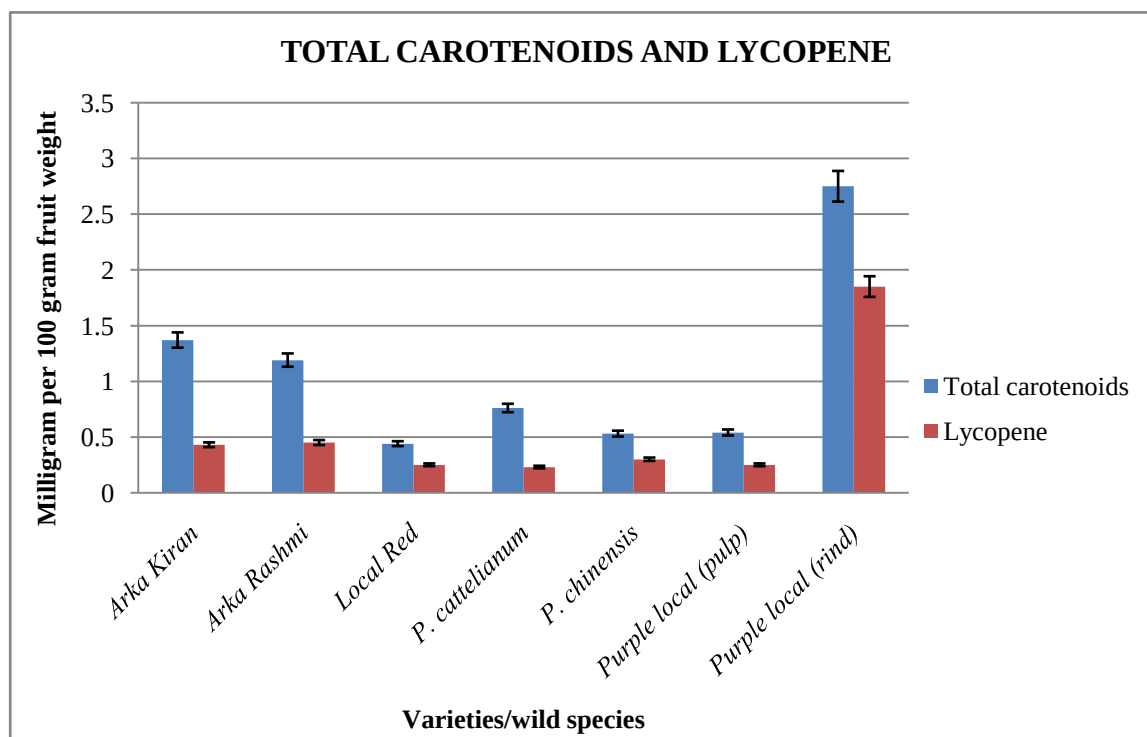


Fig. 18: Total carotenoids and lycopene of guava varieties and wild species

4.5.6 Non reducing sugar

Among the different varieties and wild species of guava, in rind Arka Amulya (3.11 %) recorded significantly highest non reducing sugar followed by Arka Mridula (3.06 %) and *P. chinensis* (2.66 %), whereas the lowest value was shown by Seedless Triploid (0.01%). In pulp, Purple Guava (4.56 %) recorded significantly the highest non reducing sugar followed by *P. guineense* (3.52 %) and Seedless Triploid (3.20 %), whereas the lowest value (1.23 %) was shown by Local Red.

4.5.7 Total carotenoids

The total carotenoids of the red fleshed varieties and wild species of guava varied significantly. The highest amount of total carotenoids was Arka Kiran (1.37mg 100g⁻¹ FW) in pulp whereas, the lowest amount of total carotenoids was shown by Local Red (Table 6).

4.5.8 Lycopene

The lycopene content of the red fleshed varieties and wild species of guava exhibited significant difference. The highest amount of lycopene was recorded in Purple local in rind (1.85 mg/100g FW) followed by *P. chinensis* (0.63 mg/100g FW) and Arka Rashmi (0.45 mg/100g FW) whereas; the lowest amount of lycopene (0.23 mg/100g FW) was recorded in *P. cattleianum*.

4.6 Volatile Aroma profiling

The data on volatile aroma profiling of different *P. guajava* varieties viz., Allahabad Safeda, Arka Amulya, Arka Kiran, Arka Mridula, Arka Rashmi, Local red, Purple Guava and Seedless Triploid and different wild species of guava viz., *P. cattleianum*, *P. chinensis*, *P. guineense* are presented in Table 7 -14, Fig. 19-25.

Table 6. Total carotenoids and lycopene content in red fleshed guava varieties and wild species

Treatments	Total carotenoids (mg 100g⁻¹ FW)	Lycopene (mg 100g⁻¹ FW)
Arka Kiran	1.37	0.43
Arka Rashmi	1.19	0.45
Local Red	0.44	0.25
<i>P. cattelianum</i>	0.76	0.23
<i>P. chinensis</i>	0.53	0.30
Purple local (pulp)	0.54	0.25
Purple local (rind)	2.75	1.85
Mean	1.08	0.54
SEm±	0.06	0.01
CV (%)	4.01	1.94
CD (P=0.05)	0.17	0.04

The results of the volatile aroma profiling carried out using the fruits at breaker stage (colour turning) in *Psidium* species and guava varieties revealed that the total compounds responsible for the aroma has fallen under eight major groups viz., hydrocarbons, monoterpenoids, sesquiterpenoids, alcohols, aldehydes and ketones, esters, oxo compounds and others. The contribution towards the total (94.26 %) fruit aroma was in the order of sesquiterpenoids (78.82%), monoterpenoids (7.39 %), hydrocarbon (2.92 %), alcohols (1.75 %), oxo compounds (1.23 %), esters (0.79 %), aldehydes and ketones (0.13 %) and others (1.23 %).

In general, irrespective of the wild species and varieties of guava, (+) longicycline (3.89 %) was the major hydrocarbon which contributes to aroma. As regards monoterpenoids, limonene (3.75 %) was major monoterpenoid, β -Caryophyllene (31.76 %) was major sesquiterpenoid, β -Bisabolol (1.71 %) was major alcohol, 1-(1,2,6,7-tetrahydro-3,8-dimethyl-5-azulenyl)-ethanone (0.22 %) was the major ketone, (Z)-valerenyl acetate (3.37 %) was the major ester, caryophyllene oxide (1.01 %) was the major oxo compound and 2,6-dimethyl-1,4-dihydropyridine-3,5-dicarbonitrile (2.6 %) was the other compound which contributes to the aroma of guava.

4.6.1 Hydrocarbon compounds present in guava varieties and wild species

The hydrocarbon compounds contributing to aroma of different guava varieties and wild species ranged from 0.25 to 7.89 %. It was highest (7.89 %) in Seedless Triploid followed by T₈- *P. Chinensis* (5.05 %) and Arka Amulya (4.55%). The least (0.25%) amount of hydrocarbon compounds was obtained in Allahabad safeda. The major hydrocarbon compound responsible for aroma of Seedless Triploid was found to be 3-pentyl-1-cyclopentene (7.42 %) and that of *P. chinensis* and Arka Amulya was found to be (+)-longicycline (4.293%) and 4- isopropyl-1,6-dimethyl-1,2,3,4,4a,7- hexahydro naphthalene (2.61 %) respectively.

In the other two species viz., *P. cattleianum* and *P. guineense*, the contribution by hydrocarbons was 3.30 and 3.64% respectively. Among the compounds, 1,1,3a-Trimethyl-7-methylenedeca-1H-cyclopropa[a]naphthalene found to be more (3.12%) in *P. cattleianum* and (+)-longicycline (3.48%) in *P. guineense*.

Table 7. Hydrocarbon compounds present in guava varieties and wild species

Compound Name	RI unit	Allahabad Safeda	Arka Amulya	Arka Kiran	Arka Mridula	Arka Rashmi	Local Red	<i>Psidium cattleianum</i>	<i>Psidium chinensis</i>	<i>Psidium guineense</i>	Purple Guava	Seedless Triploid
Cyclopentene	582								0.009			
Spiro[2.4]hepta04,6 diene	718		0.006							0.002		
Toluene	759						0.005					0.127
1,1,20Trimethylcyclopentane	795							0.001				
p-Xylene	862	0.002					0.005					0.009
3-Propyl-1-cyclopentene	885		0.018									
Styrene	895	0.001	1.807			0.038	0.057		0.007	0.003		0.108
Santolina triene	909			0.012					0.022			
(E,Z)-2,4,6-Octatriene, 2,6-dimethyl	993	0.025				0.019	0.142					0.039
1,3,5,5-Tetramethyl-1,3-cyclohexadiene	997	0.025				0.123	0.15					
p-Cymene	1026			0.048		0.014		0.002	0.169	0.003		
3-Pentyl-1-cyclopentene	1040	0.169	0.005	0.048	0.001	0.197	0.159	0.01	0.005	0.042	0.213	7.421
(3E,5E)-2,6-Dimethyl-1,3,5,7-octatetraene	1134			0.009		0.024				0.05		
2,3-Dimethyl-3-isopropyl-cyclopentene								0.051	0.547			
1,2,5,7-Tetramethyladamantane	1162			0.047							0.04	
3-Hexyl-1-cyclopentene	1168						0.002	0.013		0.026		0.002
1-Dodecene	1182	0.01				0.01						
6-Methyl-1,2,3,5,8,8a-hexahydronaphthalene	1184		0.034		0.012							
1-Dodecyne	1212	0.001				0.007	0.005					0.006

cis,trans-2-methyl-1,3-cyclooctadiene			0.001							0.019		
n-Tetradecane	1313											0.032
1,4-Di-tert-butylbenzene	1342			0.865								
Megastigma-4,6(E),8(Z)-triene	1354						0.009					
1,1,3a-Trimethyl-7-methylenedecahydro-1H-cyclopropa[a]naphthalene	1398							3.12				
4,8,8-Trimethyl-2-methylene-4-vinylbicyclo[5.2.0]nonane	1407		0.071	0.58	0.026			0.019				
AROMADENDRENE, DEHYDRO	1466									0.012		
4-Isopropyl-1,6-dimethyl-1,2,3,4,4a,7-hexahydronaphthalene	1528		2.606		4.32			0.03			0.248	
1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	1607	0.015					0.027					0.146
4-Isopropylbiphenyl	1615							0.02				
1,4-dimethyl-7-(1-methylethyl)-Azulene	1772							0.032				
(+)-Longicyclene	1373								4.293	3.483		
Total		0.248	4.547	1.609	4.359	0.432	0.561	3.298	5.053	3.639	0.501	7.891

4.6.2 Monoterpenoid compounds present in guava varieties and wild species

The monoterpenoids contributing to aroma of different guava varieties and wild species ranged from 1.14 and 21.54%. The highest contribution (21.54%) was registered by *P. chinensis* followed by Local red (14.44) and Allahabad Safeda (8.56), while lowest amount (1.14) was shown by Arka Mridula. The major monoterpenoid compound responsible for aroma of *P. chinensis* was found to be limonene (19.81%) and that of Local Red and Allahabad Safeda was found to be cis-ocimene which amounts for 8.18 and 2.02 % respectively.

P. cattleianum and *P. guineense*, the contribution by monoterpenoids was 6.60 % and 4.21 % respectively. The monoterpenoid like α -Pinene found to be more (2.37%) in *P. cattleianum* and limonene (2.61%) in *P. guineense*.

4.6.3 Sesquiterpenoid compounds present in guava varieties and wild species

The sesquiterpenoids which were the major compound contributing to the aroma of both guava varieties and wild species. It ranged from 63.19 to 87.94% among the varieties and wild species. The highest value (87.94%) was recorded by Arka Rashmi followed by Arka Kiran (85.39%) and Arka Amulya (81.20%) while the lowest value (63.19%) was recorded in *P. chinensis*.

In the wild species, the contribution by sesquiterpenoids was 79.02 and 80.15% in *P. cattleianum* and *P. guineense* respectively. Among the compounds β -Caryophyllene was found to be more (39.89) whereas, in *P. guineense*, a total of 41.108% α -Cedrene was found to be present.

4.6.4 Alcohols present in guava varieties and wild species

The alcohol compounds present in different guava varieties and wild species ranged from 0.063 to 4.42%, among which *P. guineense* recorded the highest value (4.4%) followed by *P. chinensis* (3.16%) and Arka Mridula (2.67%). The lowest value (0.06 %) was recorded in Arka Rashmi. The major alcohols contributing to aroma of *P. guineense* and *P. chinensis* was found to be β -Bisabolol (3.12 and 1.73% respectively) and that of Arka Mridula was found to be nerolidol which amounts for 1.55 %.

Table 8. Monoterpenoid compounds present in guava varieties and wild species

Compound Name	RI Unit	Allahabad Safeda	Arka Amulya	Arka Kiran	Arka Mridula	Arka Rashmi	Local Red	<i>Psidium cattleianum</i>	<i>Psidium chinensis</i>	<i>Psidium guineense</i>	Purple Guava	Seedless Triploid
Cyclofenchene	898									0.015		
α -Thujene	923			0.015					0.017			
α -Pinene	932	0.046	0.052				0.035	2.365	0.059	0.995		0.131
Camphene	946	0.001					0.003	0.006	0.001	0.003		0.005
Sabinene	972	0.001	0.002					0.077	0.036	0.002		0.003
β -Pinene	978	0.103	0.049			0.033	0.076	0.819	0.125	0.392	0.002	0.321
α -Phellandrene	1005		0.024				0.014	0.004	0.005	0.012		0.044
δ - 3-carene	1010		0.001	0.017				0.001	0.184			
α - Terpinolen	1019	0.001	0.005	0.006		0.004	0.006	0.034	0.023	0.017		0.03
Limonene	1031	5.737	4.592	0.009	0.001	0.042	4.779	0.542	19.813	2.609	0.025	3.06
trans-Ocimene	1042	0.562	0.006	0.999	0.168	0.261	1.345	0.447		0.044		1.158
cis-Ocimene	1043	2.015	1.282	0.964	0.971	2.803	8.176	2.254	1.26	0.078	5.48	3.05
γ -Terpinene	1062	0.01	0.007	0.002		0.006	0.012	0.031	0.019	0.046	0.004	0.045
Allo-Ocimene	1129	0.074				0.01		0.02				0.203
Total		8.55	6.02	2.012	1.14	3.161	14.446	6.6	21.544	4.213	5.511	8.05

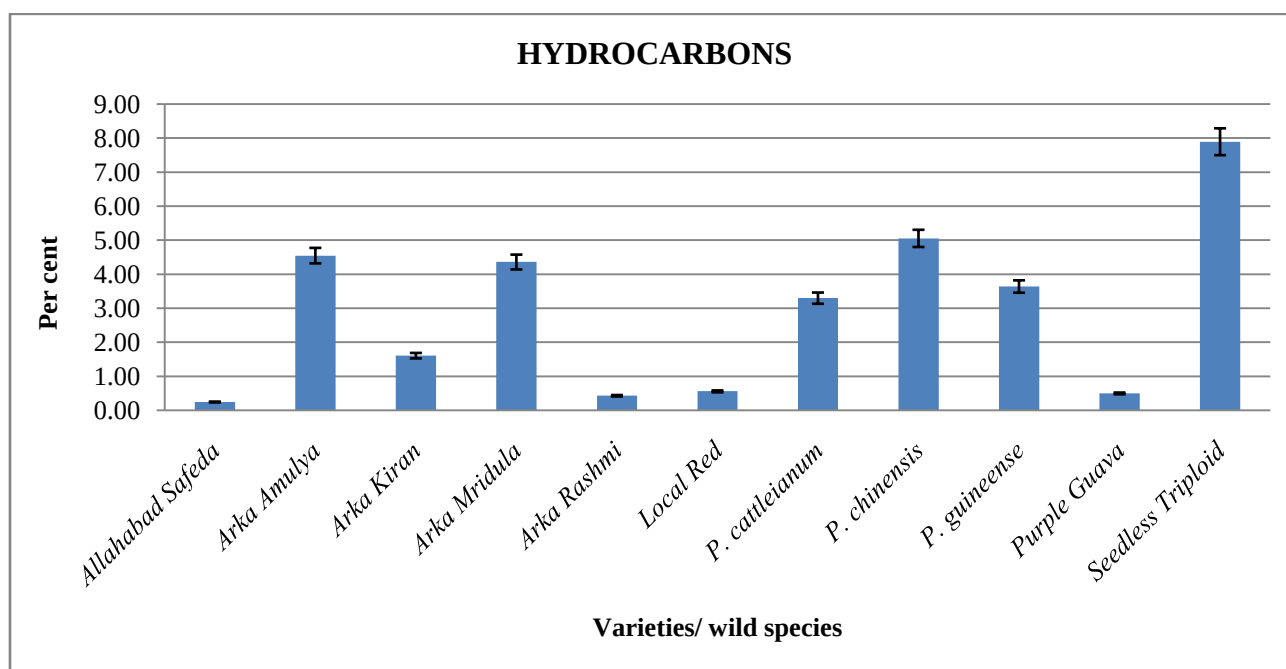


Fig. 19: Total hydrocarbons present in guava varieties and wild species

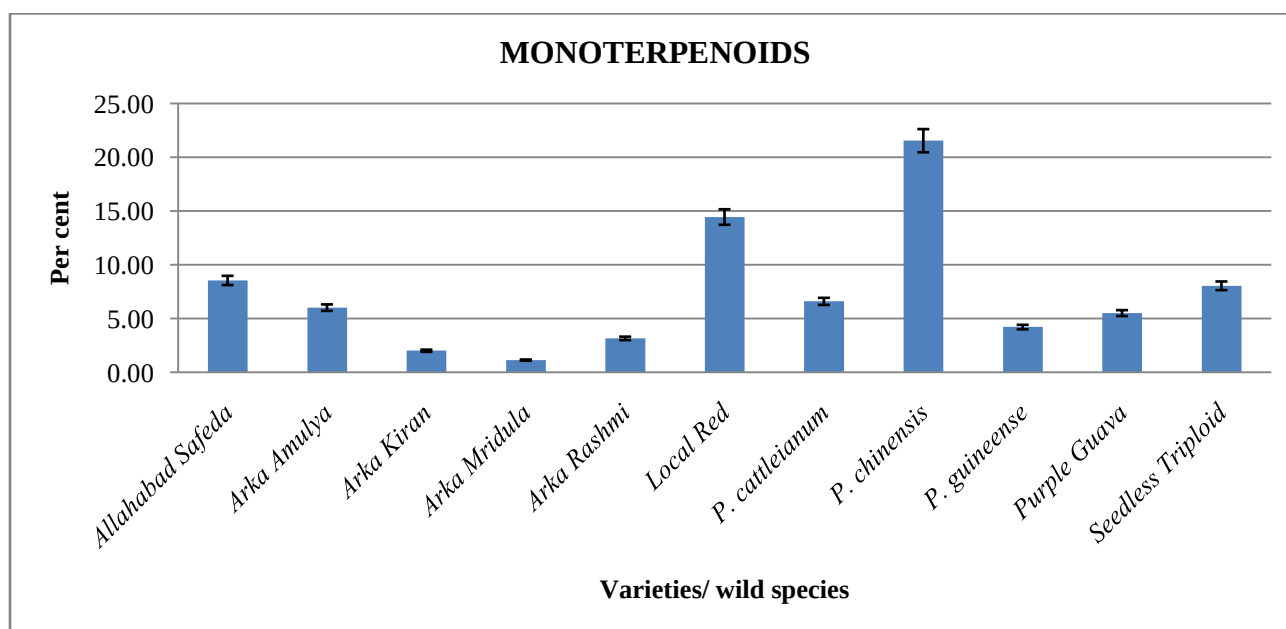


Fig. 20: Total monoterpenoids present in guava varieties and wild species

Table 9. Sesquiterpenoid compounds present in guava varieties and wild species

Compound Name	RI Unit	Allahabad Safeda	Arka Amulya	Arka Kiran	Arka Mridula	Arka Rashmi	Local Red	<i>Psidium cattleianum</i>	<i>Psidium chinensis</i>	<i>Psidium guineense</i>	Purple Guava	Seedless Triploid
(-)-Isosativene	1339			2.649								
α -Cubebene	1345	0.195	0.206	2.964	10.335	3.341	0.309	0.026	0.871	0.134	4.749	0.341
α -Longipinene	1353	0.032	0.033	0.011	0.012			0.437	3.993	3.151	3.254	0.243
(+)-Cyclosativene	1357								4.302	4.691		
α -Ylangene	1371		0.252	0.013	0.203			0.944	0.396		0.188	
Longicyclene	1378							0.209				
β -Bourbonene	1384							0.024				
α -Copaene	1392							0.46				
β -Cubebene	1393			0.255								
β -Cedrene	1394								0.115			
(-)-Aristolene	1403							0.295				
α -Gurjunene	1403							0.703				
β -Elemene	1406	0.009			0.014							
β -Patchoulene	1409									1.014		
Junipene	1413									3.8		
(Z)-CIS- α -BERGAMOTENE		5.61				1.63	3.617	0.135				3.531
α -Cedrene	1415	0.552		2.827		5.486	1.343		5.789	41.108	7.199	0.23
α -Ferulene	1416	0.023					0.148				0.01	0.076
β -Guaiane	1423			0.073				0.011	0.117			
α -Guaiane	1424		0.512		0.325	0.008				0.007	2.758	0.006
Epi- β -Santalene	1425								0.132	0.107		
Thujopsen	1430		0.015	0.065	0.206	0.114	0.023	0.012		0.009	0.205	
Elixene	1431								0.004	0.046		
β -Caryophyllene	1440	31.916	47.239	29.199	38.601	29.041	13.044	39.891	14.658		28.299	45.736
Cadinene	1440		0.142		0.199			7.023	3.325	3.697	1.566	
α -Muurolene	1440							0.273	0.085	0.297		
α -Cedrane	1441							0.014	0.546	0.56	0.92	

τ -Elemene	1445			0.072								
(+)-Aromadendrene	1447	0.017	0.373	0.147	0.271		1.025	0.203		0.054	1.272	3.15
β -Santalene	1461								1.711	1.313	2.81	
Germacrene D	1464	1.684	1.59	0.81	1.062	0.981	0.829	3.072				0.656
Germacrene B	1464							3.072				
Acoradiene	1471		0.021	2.224		3.272	1.853		2.855	1.822	3.889	
α -Muurolene	1495	1.397	0.68	0.107	10.963	0.009	0.326				0.186	0.533
α -Muurolene	1495							0.679	0.534	0.603		
α -Himachalene	1427									2.167		
β -Gurjunene	1428							0.092	0.003		0.164	
Thujopsene	1430							0.09			0.067	
τ -Gurjunene	1444			0.461					0.01		0.127	
(+)-Aromadendrene	1447							2.638				
α -Humulene	1459	4.048	5.23	5.484	4.994	2.97	2.556	4.744	3.628		2.856	4.808
α -Humulene	1459									0.452		
Allo-Aromadendrene	1461	0.912	1.102	3.784	1.12	1.937	1.312	0.501				2.028
α -Curcumene	1476		0.152	8.58	0.056	3.928	0.807				6.002	
Aciphyllene	1480								0.074	0.133		
β -Selinene	1483	0.752	0.622	0.488	0.413	3.388	0.357	0.004	4.604	3.5		1.033
τ -Muurolene	1489								2.456	1.087		
α -Selinene	1492								2.864	1.112		
Varidiflorene	1492			0.468				2.5	1.085	0.434	0.248	
(Z,E)- α -Farnesene	1496	0.088	0.103	0.696	0.013	0.539	0.157					
β -Himachalene	1499								3.29	0.795	6.659	
β -Himachalene	1499			0.209			0.375					
Zingiberene	1500	0.106		3.526		2.232	7.044				0.631	
β -Bisabolene	1501	9.161	5.801	0.83	1.254	9.339	10.488	0.734				0.305
δ -Guaiane	1505			0.021	0.033			0.062				
(-)- β -Cadinene	1508	5.61				1.63	3.617					3.531
α -Chamigrene	1512								0.009			
Cuparene	1513								5.433	4.122		

α -Bisabolene	1518	9.77	8.663	6.039	1.16	1.681	3.324		0.081			0.621
α -Bisabolene	1518									2.045	2.237	
Selina-3,7(11)-diene	1531							3.783		0.124		
τ -Cadinene	1536	2.773	1.039		1.653	7.487	8.389			0.161	0.171	0.818
τ -Cadinene	1536							1.336				
cadina-1,4-diene	1538	3.223	6.612	2.592	6.692			0.618				1.332
(-) dehydroaromadendrene	1466		0.102	0.009	0.244							
γ - himanchalene	1718	0.385										0.407
bicyclogermacrene	1494										0.245	
(-)-ISOLEDENE	1373		0.043		0.152			0.07	0.009			
α -Amorphene									0.116	0.13		
Acoradiene	1475			5.45				2.874			6.488	
γ –himanchalene	1479								0.093		0.162	
γ -curcurnine						3.866	3.062	0.208				
(+)- β -FUNEBRENE				1.445		1.617	5.286				3.463	
TRANS- τ - BISABOLENE			0.664	3.899	0.067	3.446	6.258					
α -Ferulene								1.282				
Total		78.262	81.204	85.396	80.053	87.942	75.55	79.02	63.191	80.151	86.824	69.385

Table 10: Alcohols present in guava varieties and wild species

Compound Name	RI Unit	Allahabad Safeda	Arka Amulya	Arka Kiran	Arka Mridula	Arka Rashmi	Local Red	<i>Psidium cattleianum</i>	<i>Psidium chinensis</i>	<i>Psidium guineense</i>	Purple Guava	Seedless Triploid
(2E)-2-Penten-1-ol	769						0.007					
1-Hexyn-3-ol	778						0.005			0.002		
cis-3-Hexen-1-ol	851	0.149						0.069	0.343		0.06	
1-Hexanol	860		0.044									
4,7,7-Trimethylbicyclo [4.1.0]heptan-3-ol	1135							0.027				
4-Ethyl-1,4-dimethyl-2-cyclohexen-1-ol	1147						0.004					
1-Nonanol	1160	0.011						0.003		0.006		0.063
1,Methylcyclooctanol	1171		0.011									
4-Terpinenol	1175			0.01								
α -Terpineol	1193	0.005			0.001							0.005
3-Isopropenyl020 methylcyclohexanol	1196											0.021
L-Carveol	1220	0.002			0.006		0.019			0.031		0.011
p-Benzenediol	1220							0.109				
2-Butyloctanol	1277											0.018
α ,2,5-Trimethylbenzyl alcohol	1282						0.001					
1-(2,40Dimethylphenyl) ethanol	1282						0.002					
1,2,3,4,4a,7,8,8a-octahydro-2,7-Methanonaphthalene-3-methanol	1286			0.015			0.001					
cis- α -Copaene-8-ol	1410		0.002		0.022			0.09				
cis- α -Copaene-8-ol	1410							0.124				
α -Ionol	1455								0.002			

β Ionol	1484	0.004				0.063	0.047					0.03
1-[2-(1-Hydroxyethyl)phenyl]-2-propanol	1530										0.265	
Palustrol	1530		0.056	0.094	0.002			0.069				
Nerolidol	1537	0.419	0.142		1.553		0.788					0.262
Viridiflorol	1594	0.115	0.029	0.238	0.201		0.207					0.45
α -Cedrol	1604						0.038			0.138		
Ledol	1608								0.104			
Cubenol	1642	0.013			0.022		0.077			0.025		0.048
δ -Cadinol	1646		0.041	0.013	0.29			0.018	0.797	0.621		
Cedr-8-en-15-ol	1646	0.071	0.004	0.228	0.044		0.101					0.029
Cedr-8-en-13-ol	1668	0.174	0.083	0.018	0.442		0.289		0.01			0.304
Santalol	1668			1.467			0.064					0.071
α -Santalol	1683				0.021		1.26	0.076				
β -Bisabolol	1685								1.729	3.124	0.284	
cis-Farnesol	1697								0.176			
4a-Methyl-1,2,3,4,4a,9,10,10a,octahydro010phenanthrenol	1810		0.02		0.065			0.041				
5-(1,3-dithian-2-ylidene)-4-methyl-1-penten-3-ol										0.484		
Total		0.964	0.445	2.084	2.669	0.063	2.909	0.626	3.161	4.429	0.608	1.312

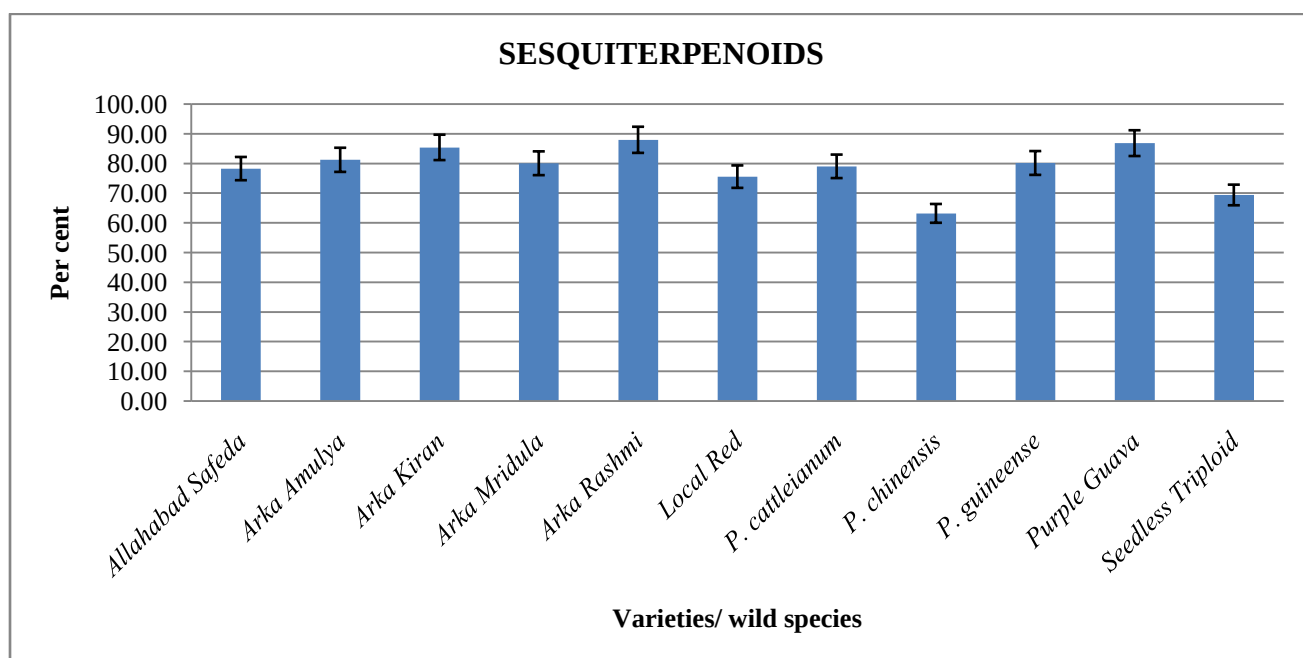


Fig. 21: Total sesquiterpenoids present in guava varieties and wild species

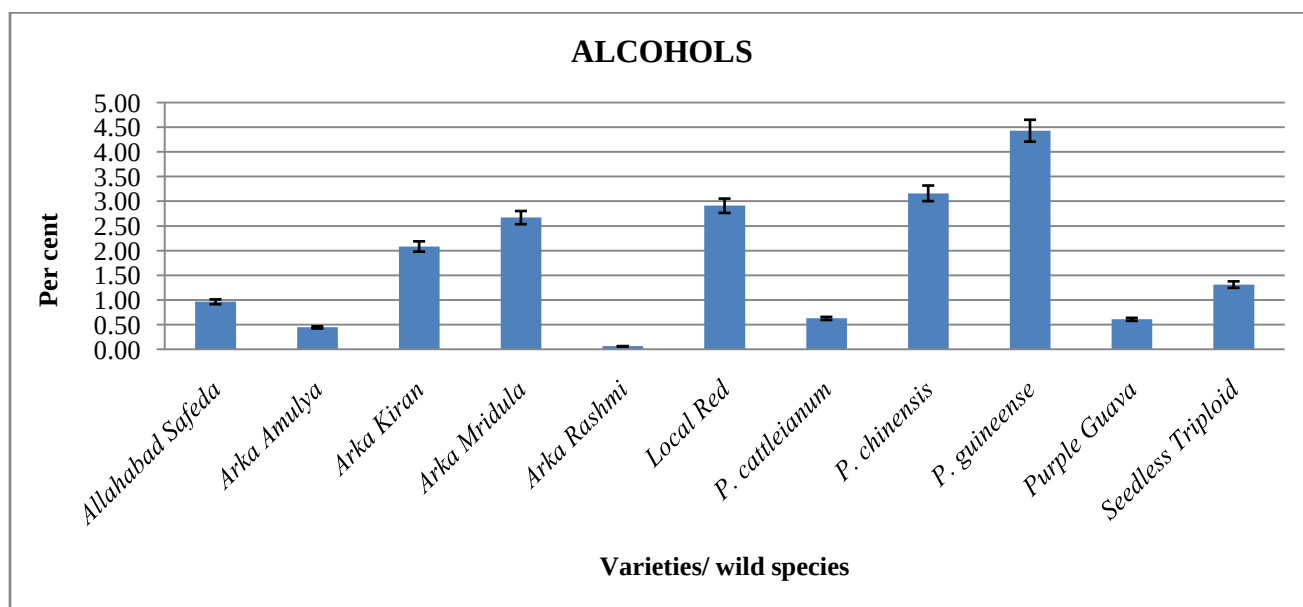


Fig. 22: Total alcohols present in guava varieties and wild species

In T₇- *P. cattleianum*, the contribution by alcohols was 0.63 % among which cis- α -Copaene-8-ol was found to be more (0.12%).

4.6.5 Aldehydes and ketones present in guava varieties and wild species

The Aldehyde and Ketone compounds contributing to aroma of different guava varieties and wild species ranged from 0.003% to 0.31 %, among which Arka Rashmi recorded the highest value (0.31 %) followed by *P. chinensis* (0.15 %) and Local Red (0.13%). The lowest value (0.003%) was recorded in *P. guineense*. The major aldehydes and ketones contributing to aroma of Arka Rashmi was found to be carvone (0.19 %) and that of *P. chinensis* and Local Red was found to be (E)-2- Hexenal which amounts for 0.08 % and 0.13% respectively.

In *P. cattleianum* and *P. guineense*, the contribution by aldehydes and esters was 0.08 and 0.003% respectively. Among various compounds, 2-Pentyl-2-cyclopenten-1-one (0.21 %) and (E)-3-Hexenal (0.003%) was found to be more in *P. cattleianum* and *P. guineense* respectively.

4.6.6 Esters present in guava varieties and wild species

The Ester compounds contributing to aroma of different guava varieties and wild species ranged from 0.016 and 3.63%. *P. cattleianum* shown the highest value (3.63%) followed by Allahabad Safeda (1.32%) and *P. guineense* (1.32%). The lowest amount (0.016%) was shown by Purple Guava. The major esters responsible for aroma of *P. cattleianum* was found to be (Z)- valerenyl acetate (3.37%) and that of Allahabad Safeda and *P. guineense* was found to be benzenepropyl acetate (1.32%) and α -Terpinyl propionate (1.05%) respectively.

In *P. chinensis* the contribution by ester was 0.01 % and the compound, α -bornyl acetate found to be more (0.01 %).

4.6.7 Oxo compounds present in guava varieties and wild species

The oxo compounds contributing to aroma of different guava varieties and wild species ranged from 0.36 to 2.95 %, among which Seedless Triploid recorded the highest value (2.95 %) followed by Arka Rashmi (2.40 %) and Arka Mridula (1.65 %). The lowest value (0.36 %) was recorded in Purple Guava. The major oxo compounds contributing to the aroma of Seedless Triploid was found to be caryophyllene oxide (2.60 %) and that of Arka Rashmi and Arka Mridula was found to be 1-(4-methoxy-2,3,6-trimethyl phenyl) ethanol (1.36 %) and caryophyllene oxide (1.64%) respectively.

Table 11: Aldehydes and ketones present in guava varieties and wild species

Compound Name	RI Unit	Allahabad Safeda	Arka Amulya	Arka Kiran	Arka Mridula	Arka Rashmi	Local Red	<i>Psidium cattleianum</i>	<i>Psidium chinensis</i>	<i>Psidium guineense</i>	Purple Guava	Seedless Triploid
(E)-3-Hexenal	860							0.004	0.002	0.003		0.038
β-Cyclocitral	1219					0.006	0.002					
Cumic aldehyde	1235			0.008	0.007							
Carvone	1240					0.186						
2-Pentyl-2-cyclopenten-1-one	1240							0.012				
(4,6,6-Trimethylbicyclo [3.1.1]hept-3-en-2-yl)acetaldehyde	1297										0.003	
4-Hydroxymethyl benzaldehyde	1338						0.001					
5-Dodecanone	1350							0.003				
2,6,6-Trimethylundeca-1,3-dien-9-yn-5-one	1457								0.071			
β-Ionone	1482										0.01	
Bourgeonal	1508										0.009	
1-(1,2,6,7-tetrahydro-3,8-dimethyl-5-azulenyl)-Ethanone	1639		0.324	0.035	0.289							
Amobarbital	1735							0.01				
Total		0.004	0.324	0.043	0.296	0.305	0.133	0.084	0.151	0.003	0.065	0.038

Table 12. Esters present in guava varieties and wild species

Compound Name	RI Unit	Allahabad Safeda	Arka Amulya	Arka Kiran	Arka Mridula	Arka Rashmi	Local Red	<i>Psidium cattleianum</i>	<i>Psidium chinensis</i>	<i>Psidium guineense</i>	Purple Guava	Seedless Triploid
Hexyl acetate	1010		0.015									
Methyl benzoate	1092						0.011					0.089
Methyl octanoate	1112					0.095	0.044					0.673
Linalyl acetate	1261							0.091				
trans-Chrysanthenyl acetate	1276	0.003	0.001	0.014	0.009	0.005	0.025					0.003
α -bornyl acetate	1284	0.001	0.002			0.006	0.003	0.003	0.01	0.012		0.018
Sabinyl acetate	1291									0.017		
(-)-cis-Carvyl Acetate	1346	0.003	0.004	0.004						0.054		
α -Terpinyl acetate	1352			0.014								
α -Terpineol acetate	1352							0.05				
Benzenepropyl acetate	1373	1.318			0.048	0.069						
α -Terpinyl propionate	1432									1.051		
3-Phenylpropyl isobutyrate	1493			0.638	0.071						0.016	
(Z)-valerenyl acetate								3.372				
Methyl 40methylene-2-phenylcyclopentanecarboxylate	1629			0.519								
Total		1.324	0.068	1.193	0.128	0.175	0.083	3.634	0.013	1.317	0.016	0.783

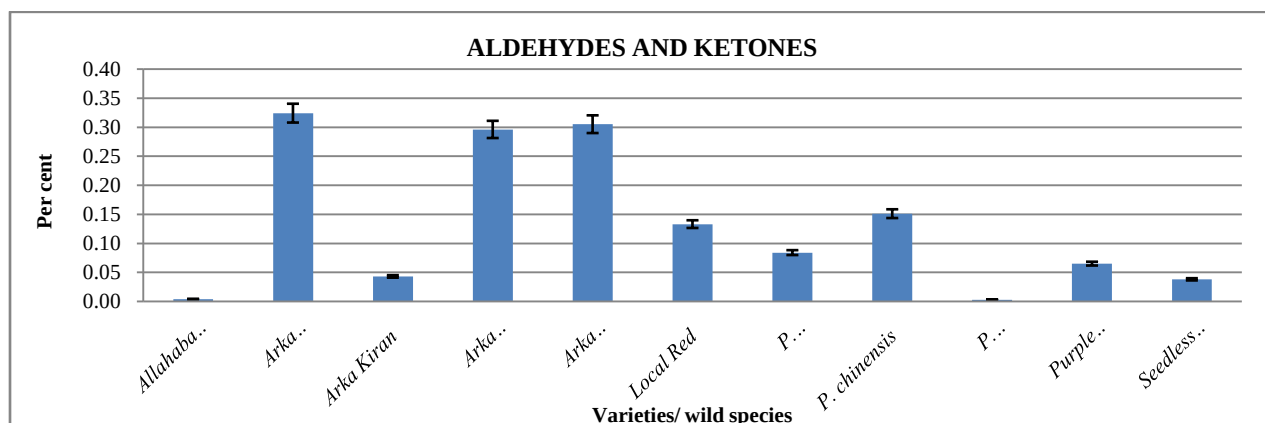


Fig. 23: Total aldehydes and ketones present in guava varieties and wild species

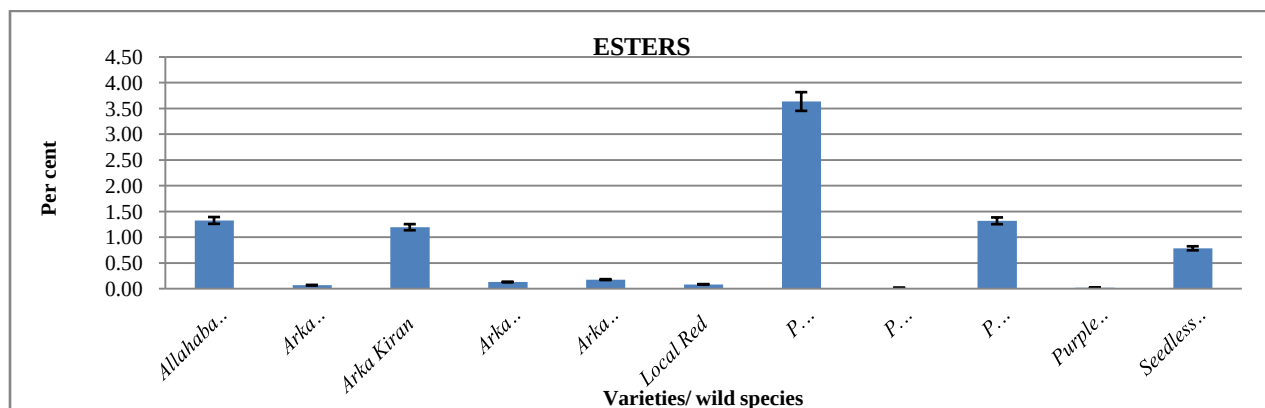


Fig. 24: Total esters present in guava varieties and wild species

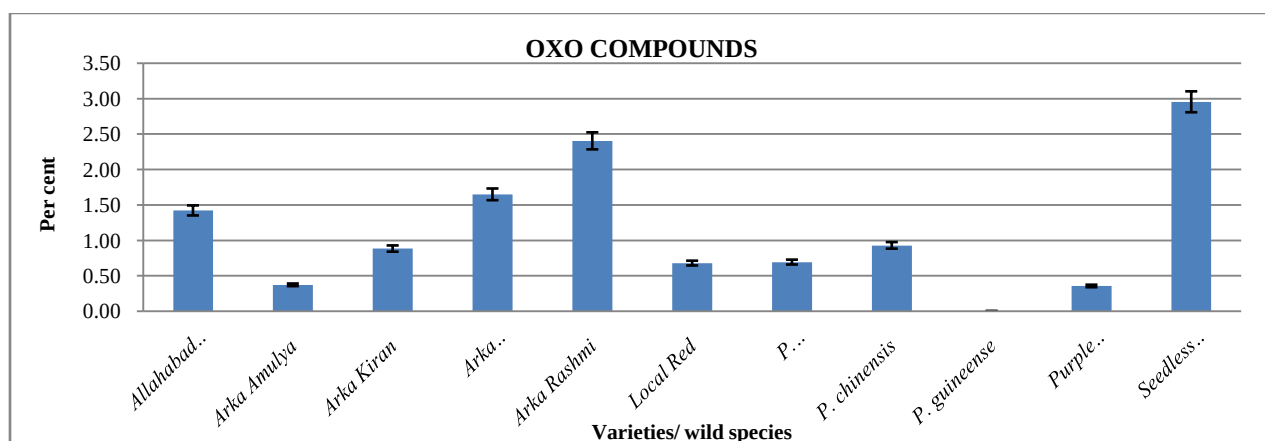


Fig. 25: Total oxo compounds present in guava varieties and wild species

In the wild species viz., *P. cattleianum* and *P. chinensis*, the contribution were 0.69 and 0.93% respectively among which, caryophyllene oxide found to be the major in both the species and was 0.69 and 0.93% respectively.

4.6.8 Other compounds present in guava varieties and wild species

The other compounds contributing to aroma of different guava varieties and wild species ranged from 0.012 to 3.727%, among which Allahabad Safeda recorded the highest value (3.73 %) followed by Seedless Triploid (4.05%) and Arka Mridula (3.58 %). The lowest value (0.01%) was recorded in *P. guineense*. The compounds contributing to the aroma of Allahabad Safeda was found to be 3-methyl-5-phenyl-1H- pyrazole (3.72 %) and that of Seedless Triploid and Arka Mridula was found to be 3-methyl-5-phenyl-1H- pyrazole (3.79 %) and 2,6-dimethyl-1,4-dihydropyridine-3,5-dicarbonitrile (3.58 %) respectively.

In the other two species viz., *P. cattleianum* and *P. chinensis*, the contribution was 0.29 % and 0.14 % among which, 3a,4,5,6,7,7a-Hexahydro-2-phenylbenzoxazole and 1-Methyl-2-pyrroleacetonitrile was found to be more (0.29% and 0.13%) in *P. cattleianum* and *P. chinensis* respectively. Whereas, 3-methyl-5-phenyl-1H-Pyrazole was found to be more (0.008%) in *P. guineense*.

4.7 Cluster Analysis based on Euclidean distance

By employing Euclidean distance, the divergence in eight varieties and three wild species of guava were assessed using 42 aroma compounds. The results are presented in Table 15 and Fig. 26.

Table 13: Oxo compounds present in guava varieties and wild species

Compound Name	RI Unit	Allahabad Safeda	Arka Amulya	Arka Kiran	Arka Mridula	Arka Rashmi	Local Red	<i>Psidium cattleianum</i>	<i>Psidium chinensis</i>	<i>Psidium guineense</i>	Purple Guava	Seedless Triploid
2-Ethyl-3-propyloxirane	769			0.016								
1-Methoxy-6,6-dimethyl-1-cyclohexene	1002											0.018
Cineole	1029			0.047	0.003	0.084					0.043	
1,4-Epidioxy-p-menth-2-ene	1238			0.005								
2-(2-methoxy-1-propenyl)-1,4-dimethyl-Benzene		0.005				0.961						0.006
Caryophyllene oxide	1567	1.268	0.347	0.814	1.644		0.513	0.692	0.928		0.312	2.598
AROMADENDRENEPOXIDE-(I)		0.149	0.018				0.166					0.324
1-(4-methoxy-2,3,6-trimethyl phenyl) ethanol			0.007		0.001	1.357						
Total		1.421	0.371	0.883	1.648	2.403	0.678	0.692	0.928		0.355	2.954

Table 14. Other compounds present in guava varieties and wild species.

Compound Name	RI Unit	Allahabad Safeda	Arka Amulya	Arka Kiran	Arka Mridula	Arka Rashmi	Local Red	<i>Psidium cattleianum</i>	<i>Psidium chinensis</i>	<i>Psidium guineense</i>	Purple Guava	Seedless Triploid
2-Pentanethiol	758					0.019	0.066					
1-Methyl-2-pyrroleacetonitrile	1111								0.129			
3-methyl-5-phenyl-1H-Pyrazole	1584	3.719								0.008		3.789
2,6-Dimethyl-1,4-dihydropyridine-3,5-dicarbonitrile	1678		1.612		3.58				0.006			
2-(Mesitylmethylene) malononitrile	1904										0.008	
3a,4,5,6,7,7a-Hexahydro-2-phenylbenzoxazole								0.278				
Total		3.727	1.612	0.006	3.58	0.019	0.066	0.286	0.135	0.012	0.053	4.047

The 42 aroma compounds which were used in the study consisted of 1,1,3a-Trimethyl-7-methylenedecahydro-1H-cyclopropa[a]naphthalene(TMC),4-Isopropyl-1,6-dimethyl-1,2,3,4,4a,7-hexahydronaphthalene (IDH), (+)-Longicyclene (Lonc), Limonene (Lim), trans-Ocimene (t-Oci), cis-Ocimene(c-Oci), (-)-Isosativene (Iso), α -Cubebene (α -Cub), α -Longipinene (α -Lonp), (+)-Cyclosativene (Cycl stvn), β -Patchoulene (β -Patch), Junipene (Jnpr), (Z)-cis- α -bergamotene (c brgmtn), α -Cedrene (α -Cdrn), β -Caryophyllene (β -Caryo), Cadinene (Cdrn), β -Santalene (β -Sntln), Acoradiene (Acrdn), α -Humulene (α -Hmln), Allo-Aromadendrene (Allo-Armdndrn), τ -Murolene (τ -Mrln), α -Selinene (α -Slnn), β -Himachalene (β -Hmchln), β -Bisabolene (β -Bsbln), (-)- β -Cadinene ((-)- β -Cdrn), Cuparene(Cupren), α -Bisabolene (α -Bsbln), τ -Cadinene (τ -Cdrn), cadina-1,4-diene (cadina-1,4-diene), Acoradiene (Acrdn), γ -curcurne (γ -curcurn), (+)- β -Funebrene ((+)- β -fnbrn), Trans- τ -bisabolene (trans- τ -bsbln), α -Ferulene (α -Frln), β -Bisabolol (β -Bsblol), Benzenepropyl acetate (Bnznprpyl actat), α -Terpinyl propionate (α -Trpnl prpiont),(Z)-valerenyl acetate ((Z)-vlrnyl actat), Caryophyllene oxide (Caryph oxd), 2,6-Dimethyl-1,4-dihydropyridine-3,5-dicarbonitrile (Dm d-d).

4.7.1 Group constellations

The eight varieties and three wild species of guava were broadly classified into two main clusters namely, cluster A and B using Euclidean distance as per Ward's method of clustering. The cluster A consist of the guava varieties viz; Arka Rashmi, Local Red, Arka Kiran, Purple guava, Arka Amulya, Allahabad Safeda, Seedless Triploid, Arka Mridula and wild species *P. cattleianum*. The cluster A was further grouped into A₁ and A₂ clusters. In cluster A₁, the varieties viz; Arka Rashmi, Local Red, Arka Kiran, Purple Guava were grouped along with wild species *P. cattleianum* and in A₂ cluster the varieties Arka Amulya, Allahabad Safeda, Seedless Triploid, Arka Mridula were clustered together. The cluster B consists of only two species viz; *P. guineense* and *P. chinensis*. The proximity matrix (Euclidean distance) presented in Table 15 revealed that Local Red and *P. guineense* were found to be more divergent (14.85) followed by Arka Kiran and *P. guineense* (14.54) while Allahabad Safeda showed close proximity to seedless triploid (7.21).

Table 15. Proximity matrix (Euclidean distance)

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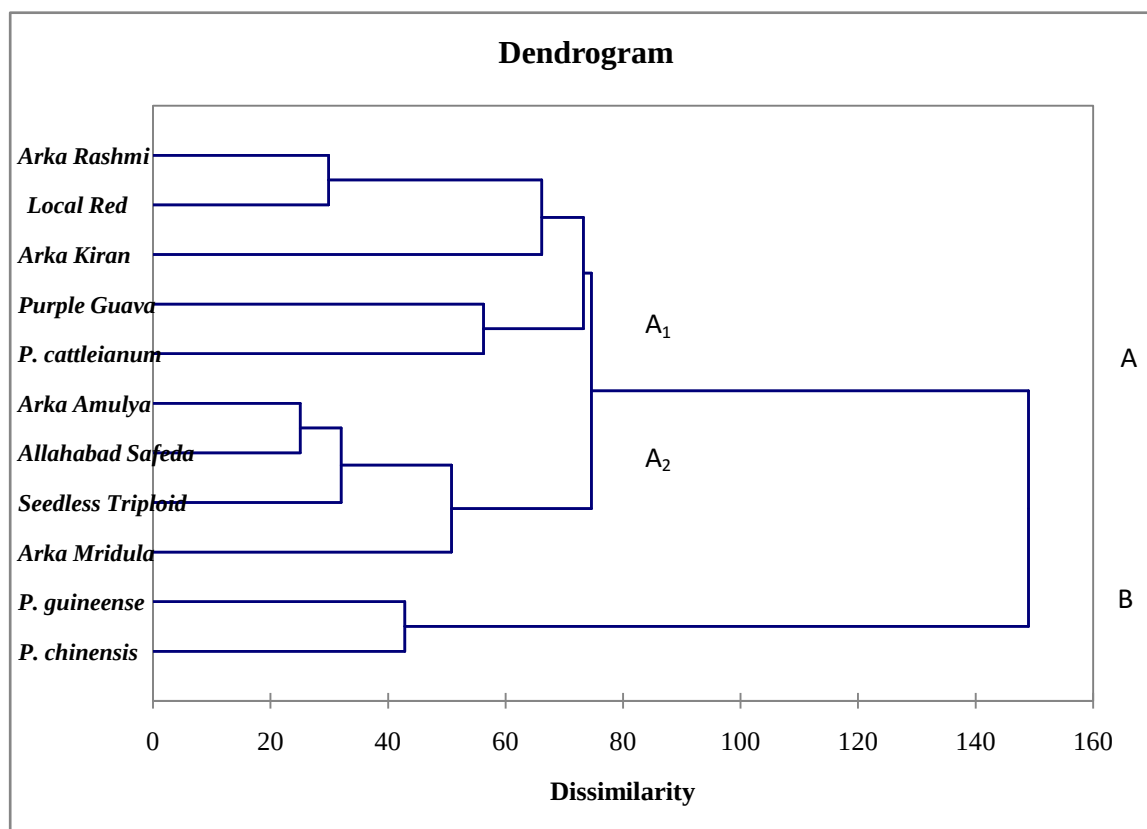


Fig. 26: Ward's method of clustering based on Euclidean distance matrix

4.8 Principal component analysis (PCA)

The spatial distribution and association among eight varieties and three wild species of guava were assessed using 42 aroma compounds by employing the principle component analysis. The same aroma compounds that were used for cluster analysis were considered for PCA. The results are presented in Fig. 27.

Out of the principal components which were estimated for eight varieties and three wild species, those components which were showing high eigen value and variability were considered for the biplot analysis. The PCA confirmed the results of cluster analysis in most of the cases. According to PCA, the varieties and species were divided into four groups consisting of wild species viz, *P. guineense* and *P. chinensis* which were closer to each other and formed a separate group, the wild species *P. cattleianum* was entirely different from this group and fallen into a separate group, red pulp varieties (Arka Kiran, Arka Rashmi and local Red) which were placed in one quarter and white pulp varieties (Arka Mridula, Arka Amulya, Allahabad Safeda and Seedless Triploid) in one separate group.

Further, the biplot analysis involving both the varieties and the major aroma compounds revealed that α -Terpinyl propionate, α -Bisabolene, β -Santalene, Junipene, α -Selinene, (+) Longicyclene, τ -Muurolene, Cuparene, β -Bisabolol, Longipinene, (+)-Cyclosativene and limonene were responsible for the unique aroma of *P. guineense* and *P. chinensis* based on which they were placed in one quarter. The compounds viz., (Z)-valerenyl acetate, 2,6-Dimethyl-1,4-dihydropyridine-3,5-dicarbonitrile, 4-Isopropyl-1,6-dimethyl-1,2,3,4,4a,7-hexahydronaphthalene, cadina-1,4-diene, τ -cadinene, α -Ferulene were responsible for unique aroma of *P. cattleianum* based on which it was placed in a separate quarter. It was also found that aroma compounds like (-)-Isosativene, Benzenepropyl acetate, β -Cubebene and Allo-Aromadendrene were found to be predominant in the guava varieties viz., Arka Kiran and Arka Rashmi.

V. DISCUSSION

Guava (*Psidium guajava* L.) is one of the most important commercial fruit crops of India. Because of its hardiest nature, it has very well adapted to a wide variety of soil and climatic condition of India and it gives assured yield with minimum care. The economic importance of guava has driven significant efforts to accelerate the exploitation of *Psidium* genetic resources for development of new cultivars towards biotic and abiotic stress improvement. The present day variability in guava genetic resources is due to the selective breeding which has started almost a century ago, cross pollination (35-40 %) which resulted in large variability in the seedling population, heterozygous nature of the crop and seed propagation in earlier days from which promising genotypes have been selected in different parts of the world.

Adequate genetic variation has been observed in *P. guajava* for the development of commercial cultivars with economic importance (Pathak and Ojha, 1993). Other *Psidium* species including *P. cattleianum* Sabine, *P. friedrichsthalianum* (O. Berg) Nied., *P. guineense* Sw., *P. montanum* Sw. and *P. sartorianum* (O. Berg) Nied are fairly well distributed and are used for crop improvement. The guava wilt is becoming a major threat worldwide and is the most devastating disease (Misra, 2006), hence, development of wilt resistant, high yielding guava cultivars deserves the first priority.

Even though source of resistance to guava wilt has been reported by Edward and Shankar (1964), the research progress is slow and needs to be hastened. The wild species viz; *P. guineense* SW, and *P. friedrichsthalianum* Niedenzu are reported to be wilt resistant (Singh *et al.* 1977). The exact causal agent of the disease is still not fully understood and needs further investigation. *Fusarium oxysporum* f. sp. *psidii* is the pathogen that causes disease in India and about 30% of the losses have been reported (Pandy and Dwivedi, 1985).

In the recent years, guava decline, a deadly disease caused due to root knot nematodes (*Meloidogyne* spp.) and *Fusarium* wilt disease complex is spreading at an alarming rate in southern and parts of central and northern India. In India, *M. enterolobii*, *M. incognita*, *M. javanica* and *M. indica* are the major root knot nematodes recorded in guava (Rao *et al.* 2017). Hence, there is an urgent need to develop efficient management strategies including the development of resistant rootstocks via breeding for introgression of resistant gene.

Psidium guineense, was known for its tolerance to the nematode, *Meloidogyne enterolobii* (Costa *et al.*, 2012). Other studies have shown that higher levels of tolerance or resistance to root knot nematodes are present in wild *Psidium* species such as *P. friedrichsthalianum*, *P. longipes*, *P. cattleianum* or *P. araca*. Some of these species have been evaluated as rootstocks in guava pointing out its compatibility and possible use (Carneiro *et al.*, 2007; Bogantes and Mora, 2008; Casassa-Padrón *et al.*, 2006). However, it is still needed to evaluate its performance under different soil and climatic conditions which occur in the different regions producing guava. According to Carneiro *et al.* (2012) Costa Rican wild guava *Psidium friedrichsthalianum*, *P. cattleyanum* (yellow wild guava), *Acca sellowiana* and *P. rufum* presented resistance or immunity to nematodes.

The Brazilian or Castilian guava (*P. guineense* (Sw). Syn: *P. molle* (Bertol), *P. araca* (Raddi), *P. schiedeanum* Berg.) withstand short period of drought. It has been used in the hybridization programme and was found to be cross compatible with the commercial varieties of guava. Still much efforts are needed to evaluate various wild species for its degree of tolerance to drought and its cross compatibility with the commercial cultivars, so as to tap its full potential.

The wild species generally contribute towards crop improvement and used as rootstock for regulation of the vigour and resistance to biotic and abiotic stresses. Hence, the two major breeding objectives are 1) to develop superior scion varieties to use as self-rooted plants (scion

breeding) 2) to evolve wilt and nematode resistant, salt tolerant and dwarfing rootstocks with wide adaptability and precocity (rootstock breeding).

In perennial crops because of the long time gap, efforts have not been made to exploit the full potential of the wild species. Although success obtained in fruit crops is low, many desirable traits with great potential for crop improvement are found in the wild species which needs to be exploited to their full potential. In order to use them in hybridization for imparting specific traits, it is imperative to study the phenological stages, chromosome number, compatibility, morphological and bio chemical traits which would help in the production of successful recombinants. And recombination breeding between varieties and wild species is one of the major approaches of guava improvement (Usman *et al.*, 2013).

Aroma profiling helps in utilizing aroma as a marker to observe the transfer of gene, as transfer of any trait may lead to change in aroma compound of the hybrid. Great deal of attention has been directed towards the characterization of the volatile profile of a wide variety of exotic fruits over the past several decades (Lasekan and Abbas, 2012).

Considering this background information and identified gaps, an attempt was made at ICAR-Indian Institute of Horticultural Research, Bengaluru to study the phenology of wild species, its crossability with *Psidium guajava* varieties, cytological studies and aroma profiling that aims at guava improvement.

5.1 Phenological stages of wild species

The study of the sequence of all periodical events involved in a plant life cycle is called 'phenology'. Plant growth rates can be evaluated by establishing the beginning and end of every developmental stage (Torres, 1995). The interval between two different phases is called 'phenological stage' (Villalpando and Ruiz, 1993). The knowledge of flower bud development, time of dehiscence and anthesis, extent of fruit set and degree of cross pollination are a pre-requisite for a planned hybridization programme.

Study on phenological stages is an important and initial step to be done in case of any crop improvement programme whenever wild species are used. This helps in understanding the flowering behavior, time and synchronization in flowering. It in turn helps to plan an effective hybridization programme. The results of the present study gives a clear understanding about the wild species on these aspects (Table 1, Fig. 1-7, Plate 8-11).

In the present study it was observed that the duration of flower bud initiation to full bloom was more in case of *P. guineense* (23 days) followed by *P. chinensis* (21) indicating that this trait could be exploited for extending the flowering duration thereby extending the fruit availability period which helps to get better market price as an early or late crop. Subramanyam and Iyer (1993) were of the opinion that the extended flowering trait of these species could be useful in the crop improvement.

The time of flowering (February-April and August-September) observed in the wild species viz., *P. guineense* and *P. chinensis* was in synchrony with the commercially cultivated species *P. guajava*. Hence, it is a favorable trait for exploitation of the specific character from the wild species, where hybridization can be effected with fresh pollen, thereby increasing the chances of good fruit set and seed recovery. Use of fresh pollen for higher fruit set and seed recovery in mango has been reported (Vasugi *et al.*, 2007).

Differential flowering time (June) noticed in the wild *P. cattleianum* could be due to the inherent genetic makeup of the species, where the possibility of pollen storage could be

attempted for introgression with the commercial varieties. Ray (2002) has also recorded similar results, where, in India, the duration of flowering during two peak flowering seasons was noticed in 35 to 45 days respectively in *P. guajava* varieties.

The duration of flowering was the highest (65) for Arka Kiran followed by *P. guineense* (60) and *P. chinensis* (58). Duration was the lowest (30) for *P. cattleianum*. This might be due to the inherent species behavior. According to Seth (1963) maturation of floral buds and fruits was most rapid in *P. cattleianum* which is in agreement with the present results. Knowledge about duration of flowering in cultivars, which gives extended fruiting, will help to avoid glut in market that will result in premier price in market.

Anther dehiscence recorded in the study was from 7.00 to 9.00 a.m in the commercially cultivated guava variety as well as the wild species viz., *P. guineense* and *P. cattleianum*. In *P. chinensis*, little delay in the flower opening was noticed (8.00 to 10.00 a.m). The synchrony of anther dehiscence of the wild species with the commercially cultivated species is a welcoming trait as it helps in the transfer of fresh viable pollen grains which in turn helps in good fruit and seed set. The results are in agreement with the findings of Ray (2002) where he found that anthesis starts at 4.00 a.m. and continues till 10.00 a.m., the peak opening occurring between 5.00 and 7.00 a.m.

In the present investigation, it was observed that the percent pollen germination was highest (80.34%) in Arka Kiran followed by *P. chinensis* (65.46%) and *P. guineense* (62.30%). The good germination indicates the ability of the pollen in effecting successful crosses. The encouraging results obtained by wild species indicate its potential for crossing success. The low germination (40.23%) exhibited by *P. cattleianum* could be due to different behavior of the species. Seth (1963) reported that *P. cattleianum* pollen had the lowest viability which is in accordance with the present study. Whereas, Hirano and Nakasone (1969) reported that the pollen of *P. cattleianum* var. *lucidum* could not be germinated.

Duration of flowering to fruit set was low (12) in the wild species *P. cattleianum* compared to the guava cultivars and other wild species used in the study. The lesser duration of flowering to fruit set is advantageous in obtaining early crop thereby helping in premium price for the produce. Seth (1963) obtained similar results in his study on *P. cattleianum*. He reported that maturation of floral buds and fruits was most rapid in this species which is in accordance with the present study.

In the present study, duration of fruit set to harvest was observed to be shortest (100) in *P. cattleianum* as compared to Arka Kiran (120), *P. chinensis* (120) and *P. guineense* (103). This trait is of importance in getting an early crop by reducing the time taken for the fruit development to attain early harvestable maturity. These results were supplementary with the findings of Garce's (1987), Barcelo' *et al.* (1992) and Coombe (1976). According to Mercado-Silva *et al.* (1998) the guava cultivar, 'Media china' also showed similar results, where the spring–summer crop took lesser days (130 days) to develop while autumn–winter crop required more (190) days to reach the ripe stage.

In the present study, the duration of fruiting recorded was the longest (120) in Arka Kiran followed by *P. guineense* (80) and *P. chinensis* (70) compared to *P. cattleianum* which showed the shortest (65) duration of fruiting. The longest duration of fruiting is of very much importance in the production of extended crop availability which in turn will help to avoid glut in market and to get premium market price. As per the findings of Normand *et al.* (2002) the fruiting lasted upto 1.5 to 2 months in *P. cattleianum* which is in accordance with the present study.

5.2 Interspecific hybridization using wild species

Utilization of wild species for crop improvement has been one of the ways to introduce certain gene(s) for specific purposes like hardness, disease and pest resistance etc through interspecific hybridization. In the present study the wild species utilized for hybridization showed significant difference for various traits studied (Table 2, Fig 8-10, Plate 12).

In the present investigation it was observed that the mean per cent fruit set was significantly high (97.07%) in the treatment combination Arka Rashmi $\times$ *P. chinensis* (Nutrient Solution) followed by Arka Rashmi $\times$ *P. chinensis* (Control) which recorded 89.94% fruit set and Arka Mridula $\times$ *P. chinensis* (Nutrient solution) with 88.62 %. The lowest (32.00%) per cent fruit set was recorded in the treatment combination of Arka Kiran $\times$ *P. guineense* (Control). The result obtained shows that *P. chinensis* is a good combiner with the cultivated variety Arka Rashmi. According to Pereira and Nachtigal (2002), the first step in crop improvement work is the selection of the parents, in order to make possible the combination of favourable characters in the descendants to be selected. It was also observed in the present study that enhanced fruit set was realized by the use of nutrient solution. Similar results of enhanced fruit set was obtained in the intergeneric hybridization of papaya (Jayavalli *et al.*, 2011). Work on interspecific hybridization involving Arka Mridula $\times$ *Psidium guineense* recorded 76.99% fruit set followed by Arka Kiran $\times$ *P. guineense* with a fruit set of 68.66 % at IIHR, Bengaluru (Das *et al.*, 2016).

5.2.1 Number of fruits

The number of fruits harvested was significantly high (15) in the combination Arka Kiran $\times$ *P. chinensis* (Nutrient solution) followed by Arka Kiran $\times$ *P. chinensis* (Control) with 10 fruits. The next best combination was Arka Rashmi $\times$ *P. chinensis* (Nutrient solution) which resulted in the recovery of 9 fruits as compared to other combinations. Similar to fruit set, the species *P. chinensis* was found to be the good combiner with the cultivated species in effecting good fruit recovery. The good percentage of viable pollen recorded in this species could have contributed to good fruit set and recovery. Also, the variations obtained could be explained due to the varying number of flowers crossed and number of flowers carried to final maturity in various combinations. Similar variations were obtained by Jayavalli *et al.* (2011) in intergeneric hybridization of papaya.

5.2.2 Number of seeds

In the present investigation, the number of seeds obtained under various treatment combinations differed significantly and was in the range of 6758.67 to 456.67. The highest (6758.67) number of seeds was obtained from the treatment combination of Arka Kiran $\times$ *P. chinensis* (Nutrient solution) followed by Arka Kiran $\times$ *P. chinensis* (Control) with a recovery of 5495.00 seeds and Arka Rashmi $\times$ *P. chinensis* (Nutrient solution) with 4211.67 seeds. This could be due the high pollen viability, highest fruit set and number of fruits harvested in these combinations which again confirms that *P. chinensis* is a good combiner. Jayavalli *et al.* (2011) was also of the opinion that the number of seeds recovered varied as per the combinations in intergeneric hybridization of papaya.

5.2.3 Per cent germination

The seedling germination obtained in the interspecific crosses was in the range of 2.8 to 99 per cent. The highest (99) per cent germination of seedlings was obtained from the treatment combination of Arka Rashmi $\times$ *P. chinensis* (Nutrient solution) followed by Arka Kiran $\times$ *P. guineense* (Nutrient solution) with a germination percent of 98.21 and Arka Rashmi $\times$ *P. guineense* (Control) with 92.85. From the overall germination results obtained, all the

combinations showed good germination except Arka Mridula $\times$ *P. chinensis* combination. The good seedling germination obtained in this study under various combinations explains the successful crosses of interspecific hybridization for guava improvement.

Hybrids from interspecific crosses are easily detectable morphologically because of the distinct morphological characters of the parents. Existence of natural hybrids between *P. guajava* and *P. guineense* were reported in Latin American regions through morphological and biochemical traits (Landrum *et al.*, 1995). Hybridization was done between *P. molle* and *P. guajava* at CISH, Lucknow. The interspecific hybrids have been found resistant to guava wilt and are graft compatible with commercial varieties of *P. guajava* and being evaluated for its wilt resistance (Anon., 2003-04).

5.3 Cytological study in *Psidium* species

The cytological study (Plate 13) carried out using root tips obtained from young sprouts of *P. guajava* cvs (Arka Amulya, Arka Kiran, Arka Mridula, Arka Rashmi, Local Red, Purple guava, Seedless triploid and Thailand guava) and the wild species (*P. cattleianum*, *P. chinensis* and *P. guineense*) could not reveal the clear active mitotic metaphase stage for the exact chromosome count which needs further standardization. However, it was reported (Coser *et al.*, 2012) earlier that guava genotypes have a stable, very small diploid genome ($2n = 2x = 22$; $2C = 0.95$ pg). Soubihe Sobrinho, (1951), Kumar and Ranade, (1952) were also of the same opinion ($2n = 22$). As regards species, the chromosome number of *P. cattleianum* was reported as $2n = 77$ and *P. guineense* as $2n = 44$ (Hirano, 1967).

5.4 Morphological traits of guava varieties and species

Morphological traits help to know about the characters of each species and cultivated varieties used in the study, thereby selection of species based on specific traits for introgression into the cultivated varieties are possible through hybridization. The results obtained in the present study revealed wide variation (Table 3, Fig 11-13, Plate 14-15).

5.4.1 Fruit traits

It was observed in the present investigation that there exists a wide variation (22.90 to 196.67 g, 15.2 to 170 ml, 2.90 to 5.93 cm, 3.00 to 5.80 cm and 1.31 to 0.45 cm respectively) for the fruit traits (fruit weight, fruit volume, fruit length, fruit width and thickness of outer pulp in relation to core diameter) among different species and varieties of guava. Among the varieties, Allahabad Safeda has recorded the highest value for all traits *viz.*, fruit weight (196.67 g), fruit volume (170.00 ml), fruit length (5.93 cm) and fruit width (5.80 cm) except for thickness of outer pulp in relation to core diameter for which Arka Mridula (1.31 cm) has recorded higher values compared to other. It is clear from the study that the variety Allahabad Safeda continues to be the most popular commercial variety of India due to these desirable traits. The variations observed in the present study could be explained due to the inherent genetic makeup of the varieties and species. A similar result was reported by Singh (1988) that there was a varietal variation for fruit weight, length, breadth, seed content, TSS and acidity.

As regards fruit shape, considerable variation was observed among the varieties and species studied. As the visual appearance of fresh fruit is the one of the first quality determinants adopted by the consumer, fruit shape is one of the important traits from the marketing point of view. The most preferred shape is globose. Among the varieties and species studied, the fruit shape was round or globose for all varieties and wild species except Local Red and *P. guineense* which produced sub-globose fruits. It was oblong in Seedless Triploid. Sharma *et al.* (2010) studied similar variations in the fruit shape among the different genotypes studied, which varies

from ovate, obovate, round, oblate, pear shaped, oblong, and oval and this results is in confirmation with the present findings.

With respect to pericarp/peel colour, considerable variation viz., yellow white, grey yellow, red purple and red were recorded among the varieties and species studied. Allahabad Safeda, Arka Amulya, Arka Mridula, *P. chinensis* and Seedless Triploid were recorded to be yellow white whereas, that of Arka Kiran, Arka Rashmi, Local Red and *P. guineense* were recorded as grey yellow. It is interesting to note that the peel colour of *P. cattleianum* was found to be red and that of Purple guava was found to be purple even after ripening. For imparting this novel trait of red and or purple colour to the progenies, these varieties may be useful as gene donor. A study conducted on 16 morphological characters on 20 genotypes of guava reported similar variations in the fruit peel colour among the different genotypes studied, which includes green, yellowish green, greenish yellow, green with reddish blush etc (Sharma *et al.*, 2010) and this results is in confirmation with the present findings.

5.4.2 Pulp and Seed traits

With respect to pulp and seed traits viz., core diameter, 100 seed weight, seed number, seed hardness and pulp colour significant variation was observed and ranged from 2.80 to 4.80 cm, 0.06 to 1.07 g, 19.0 to 363 and 8.0 to 10.83 kg cm⁻² respectively was noticed among the varieties and species. The character seed hardness is very much important in guava as we are consuming the whole fruit along with seeds. Hence, developing varieties with soft seeds is a welcoming attribute in guava improvement. As regards wild species, *P. chinensis* was found to have soft (5.00 kg cm⁻²) which could be exploited in guava improvement. Similar variations were obtained by earlier guava workers (Mitra *et al.*, 1983, Vasugi and Dinesh, 2007).

Although diploid commercial cultivars of guava have resulted in production of quality fruit, yet high seed content restricts its consumption and spread worldwide (Bosco *et al.*, 1992). Thus developing fruits with few and soft seeds have attracted the attention of breeders and researchers globally and is an important priority thrust area of the guava breeding program (Raza *et al.*, 2003; Negi and Rajan, 2007; Dinesh and Reddy, 2001; Rajan *et al.*, 2005, Dinesh and Vasugi, 2010).

5.5 Biochemical traits

The biochemical traits (TSS, Titrable Acidity, Vitamin C, Total, Reducing and Non-reducing sugars varied significantly among the varieties and wild species (Table 5, Fig. 14-17).

As expected the TSS (⁰B) was found to be high (11.87) in the commercially cultivated species viz., *P. guajava* as compared to the wild species. This is because of the superiority nature of the genotype. The TSS of the different species and cultivars of guava studied ranged from 7.73 to 11.87⁰B which was in accordance with the study conducted by Vasugi and Dinesh (2007).

As titrable acidity is a measure of fruit quality, proper sugar acid blend influences the taste and flavour of the fruit. In the present study, the titrable acidity was found to be low (0.597%) in *P. guineense* which is comparable with cultivated species. This species (*P. guineense*) is widely exploited in the preparation of several post-harvest products which could be explained due to the proper sugar acid blend. Similar results were obtained by earlier workers (Vasugi and Dinesh, 2007).

Guava is popularly called as poor man's apple due to its rich vitamin C (100-260 mg 100g⁻¹) content in addition to minerals. It was observed in the present investigation that both wild species *P. cattleianum* (242.53 mg 100g⁻¹) and *P. chinensis* (232.47 mg 100g⁻¹) were found to have high ascorbic acid and are on par with the cultivated varieties. Apart from the use of wild

species in the processing industries, the species *P. chinensis* could also be used for table purpose. The result obtained in this study was in accordance with the findings of earlier workers (Vasugi and Dinesh, 2007).

The total sugar is one of the parameters which decides the fruit quality attribute. In the present study, the total sugar recorded was found to be high (7.52 %, 8.56 % respectively in rind and pulp) in the wild species *P. chinensis* and Purple guava (7.10%, 9.04%) which could be exploited in the improvement programme. In white and pink seedling varieties of guava, the main sugar components were fructose and sucrose, which are good with respect to the health point of view (Mowlah and Itoo, 1982).

The total carotenoid is one of the important trait which decides the nutritional quality of the fruit. It was observed in the present study that the variety purple local has recorded highest amount of total carotenoids in the rind (2.75 mg 100g⁻¹ FW) followed by Arka Kiran (1.37 mg 100g⁻¹ FW) (Table 6, Fig 18). It is very interesting to note that this trait has been inherited in to the hybrids Arka Kiran and Arka Rashmi where, purple local has been used as one of the parents. The trait lycopene also followed similar trend with highest in purple local rind (1.85 mg 100g⁻¹ FW) followed by Arka Rashmi (0.45 mg 100g⁻¹ FW). Jain *et al.* (2003), recorded similar results in guava cultivar Banarsi Surkha where the total carotenoid recorded was 0.42 mg 100 g⁻¹ cultivars with substantial concentrations of antioxidant compounds, such as vitamin C, carotenoids and phenolics (Chorilli *et al.*, 2007), have become one of the major priorities of crop breeding programs (Carvalho *et al.*, 2006).

5.6 Volatile Aroma profiling

The wild species are rich source of aromas with unusual sensory properties that can attract the interest of consumers. Hence, great deal of attention has been directed towards the characterization of the volatile profile of these species.

The volatile aroma profiling of different *P. guajava* varieties viz., Allahabad Safeda, Arka Amulya, Arka Kiran, Arka Mridula, Arka Rashmi, Local red, Purple Guava and Seedless Triploid and different wild species of guava viz., *P. cattleianum*, *P. chinensis*, *P. guineense* carried out using the fruits at breaker stage (colour turning) revealed that the total compounds responsible for the aroma contribution towards the total (94.26 %) fruit aroma was in the order of sesquiterpenoids (78.82%), monoterpenoids (7.39 %), hydrocarbon (2.92 %), alcohols (1.75 %), oxo compounds (1.23 %), esters (0.79 %), aldehydes and ketones (0.13 %) and others (1.23 %) (Table 7-14, Fig 19-25). Thus the 197 aroma compounds obtained in this present investigation were classified into eight major groups.

The cultivars and wild species of guava have shown considerable difference in the aroma compounds present in them. This could be considered as one of the reasons for the different aroma of these cultivars and species. The aroma profiling of cultivated and wild species helps in understanding the transfer of gene from wild species to cultivated species as these aromatic compounds may be have linkage with other desirable genes, thus it could serve the purpose of a marker. Moreover, the specific aroma of various wild species can be transferred to the cultivated species through hybridization. It will also help in developing new varieties with specific aroma which can be directly used in processing industry which can replace the use of synthetic aroma compounds. As such processed products are having health benefits; it increases the consumer preference thus enhancing the value of the crop.

The hydrocarbon compounds contributing to aroma of guava was in the range of 0.25 (Allahaba Safeda) to 7.89 % (Seedless Triploid) in the varieties. Among the wild species, *P. chinensis* (5.05 %) was found to have higher hydrocarbon content followed by *P. guineense* (3.64 %) and *P. cattleianum* (3.30 %). Compared to other wild species, the fruits of *P. chinensis* are

firm and tastes similar to *P. guajava* in eating quality. The variation observed in the present study could be due explained to the inherent varietal characters of the species and varieties selected in this study. Even though hydrocarbons do not contribute much to the aroma, the results obtained in this study showed higher percentage of hydrocarbons contributing to aroma which could be explained due to the matured breaker stage of fruits taken for aroma profiling. Thus, the present study explained that hydrocarbons are the major aroma compounds at the colour break stage of guava fruit, which is the preferred stage of relishing as a fresh fruit.

The monoterpenoid compounds contributing to aroma of guava was in the range of 1.14 (Arka Mridula) to 21.54 % (*P. chinensis*) in the varieties. The amount of monoterpenoid limonene was found to be highest in *P. chinensis* (19.81%) and *P. guineense* (2.61%) which gives specific lemon, fruity, herbal, peppermint flavor (Chen *et al.*, 2006). This specific aroma could be effectively transferred to the cultivars through hybridization which could help to increase the consumer preference for this specific aroma.

The Sesquiterpenoid compounds contributing to aroma of guava was in the range of 63.19 (*P. chinensis*) to 87.94% (Arka Rashmi) in the varieties. β -Caryophyllene was found to be high in the wild species as well as in the cultivar which gives a woody, fruity and sweet aroma (Chen *et al.*, 2006). The wild species, *P. guineense*, revealed that α -Cedrene (41.11%) as a predominant compound, which could be exploited in the crop improvement for imparting the processing traits. In a study conducted on guava puree by Wilson and Shaw (1978), reported that β -caryophyllene was the major aroma contributing compound in guava. According to Chen *et al.* (2006), in *Psidium guajava* L. cv. Chung-Shan-Yueh-Pa, β -caryophyllene was found to be the major compound contributing to aroma of guava, which supports the observation of the present study.

The alcohol compounds contributing to aroma of guava was in the range of 0.06 (Arka Rashmi) to 4.42 % (*P. guineense*) in the varieties. The specific alcohol β -Bisabolol was found to be present in higher quantity in *P. guineense* (3.12 %) and in *P. chinensis* (1.73 %), and cis- α -Copaene-8-ol in *P. cattleianum* (0.12%) which contributes to the specific aroma. Thus these wild species could be potential donors for these compounds. In the cultivars used for this study, α -Santalol (0.64%) was found to be highest followed by Nerolidol (0.63%). In a similar study conducted by Chen *et al.* (2006) on *Psidium guajava* L. cv. Chung-Shan-Yueh-Pa, that in guava fruits, sesquiterpene alcohols amounted nearly 21% of the total volatiles, and included nerolidol as a major compound. In a study conducted by Paniandy *et al.* (2000) nerolidol was reported as the major volatile constituents in the guava fruit.

The aldehyde and ketone compounds contributing to aroma of guava was in the range of 0.003 (*P. guineense*) to 0.32% (Arka Amulya) in the varieties. The amount of aldehydes and ketones present in wild species was at par with cultivars. The major aldehyde and ketone compounds present in wild species were 2,6,6-Trimethylundeca-1,3-dien-9-yn-5-one in *P. chinensis* (0.07%), 2-Pentyl-2-cyclopenten-1-one in *P. cattleianum* (0.012%) and (E)-3-Hexenal in *P. guineense* (0.001%). As explained earlier, the stage of fruits selected for this study was at the colour break stage, due to which the contribution by aldehyde and ketone to the total aroma was less. In a study conducted by Egea *et al.* (2014) on the comparative analysis of aroma compounds and sensorial features of strawberry and lemon guavas (*Psidium cattleianum* Sabine), (Z)-3-hexenal was the major compound which gives aroma to guava fruits, which is not in accordance with the present study.

The ester compounds contributing to aroma of guava was in the range of 0.01 (*P. chinensis*) to 3.63 % (*P. cattleianum*). The present study revealed that amount of esters which contributes to the typical fruity flavor was found to be considerably less during the colour break stage. Variation in aroma compounds in wild species and varieties could be explained due to the inherent genetic makeup of the species and varieties. In the present study, amount of esters present in the wild species was found to be at par with the cultivars. Among all the varieties and

species, *P. cattleianum* was found to have highest concentration of esters. The specific ester (Z)-valerenyl acetate in *P. cattleianum* (3.37%), α -bornyl acetate in *P. chinensis* (0.01%), α -Terpinyl propionate in *P. guineense* (1.05%) are the major aroma compounds. Thus, these species could be utilized to transfer the specific aroma compounds to the cultivated species through hybridization.

The oxo compounds contributing to aroma of guava was in the range of 0.36 (Purple Local) to 2.95% (Seedless Triploid) in the cultivars and wild species of guava. This could be explained to the genetic makeup of species and varieties. In wild species, the only oxo compound present was caryophyllene oxide, which was in the range of 0.93 in *P. chinensis* and 0.69% in *P. cattleianum*. This gives a sweet, fruity, carrot aroma to the fruits, which could be utilized to transfer this aroma compound. The other compounds contributing to aroma of guava was in the range of 0.006 (Arka Kiran) to 4.05 % (Allahabad Safeda) in the cultivars and wild species of guava. The compounds like 3a,4,5,6,7,7a-Hexahydro-2-phenylbenzoxazole in *P. cattleianum* (0.28%), 1-Methyl-2-pyrroleacetonitrile in *P. chinensis* (0.14%) and 3-methyl-5-phenyl-1H-Pyrazole in *P. guineense* (0.012%) are specific to these species.

The cluster analysis (Table 15, Fig. 26) was carried out to distinguish the possible genotypes using Ward's method. It resulted in the subdivision of eight varieties and three wild species into two major clusters i.e, cluster A and B. The cluster A consist of nine accessions (Arka Rashmi, Local Red, Arka Kiran, Purple guava, *P. cattleianum*, Arka Amulya, Allahabad Safeda, Seedless Triploid and Arka Mridula) and cluster B consist of two wild species (*P. guineense* and *P. chinensis*).

The major aroma compound viz; α -Terpinyl propionate, α -Bisabolene, β -Santalene, Junipene, α -Selinene, (+) Longicyclene, τ -Muurolene, Cuparene, β -Bisabolol, Longipinene, (+)-Cyclosativene and limonene which were found to be high in *P. guineense* and *P. chinensis* made these two accessions to group together. They are very unique to these wild species which contributes to the aroma.

In study conducted by Rahimmalek *et al.* (2017) on essential oil variation in Iranian Ajowan (*Trachyspermum ammi* (L.) Sprague), the populations can be classified into two chemotypes according to hierarchical cluster and principal component analysis. These chemotypes included high thymol, and high Y-terpinene and p-cymene groups. Accordingly the results obtained in the present study could also be made useful in classifying parents and hybrids based on the unique aroma compound.

It was observed from the A₁ cluster that the guava varieties like Arka Rashmi and Arka Kiran have been clustered with Local Red and Purple Local which indicates that aroma compounds viz; Benzenepropyl acetate, α -Bisabolene, Allo-Aromadendrene, trans-Ocimene, (-)- β -Cadinene, (Z)-cis- α -bergamotene, cis-Ocimene, β -Bisabolene, Trans- τ -bisabolene, (+)- β -Funebrene, γ -curcurnine, τ -Cadinene are dominant in these red fleshed varieties. Further, it is clear from the grouping that the variety Arka Rashmi and Arka Kiran were grouped with Purple Local, which is one of the parents. These cultivars share the common aroma of (-)-Isosativene, β -Cubebene, Benzenepropyl acetate, Acoradiene. This could be used as one of the selection indices to determine the parents and hybrids. As expected aroma profiling could be exploited as a marker to determine the parents and hybrids in this study.

Similarly, the A₂ sub cluster consists of white pulp varieties viz; Allahabad Safeda, Seedless Triploid, Arka Mridula and Arka Amulya. It is very interesting to note that the aroma of white pulp varieties is mainly contributed by Cedr-8-en-13-ol, Nerolidol, 3-Phenylpropyl isobutyrate, 3-Pentyl-1-cyclopentene, α -Bisabolene, Viridiflorol, α -Cubebene, α -Humulene. Further, it is very interesting to note that the variety Arka Mridula and Arka Amulya are grouped

together with their parents Allahabad Safeda and Seedless Triploid. Hence, as expected aroma profiling could be used as a marker to detect the progenies.

In principal component analysis also the wild species *P. guineense* and *P. chinensis* formed a separate group similar to that of cluster analysis which was mainly based on unique aroma compounds α -Terpinyl propionate, α -Bisabolene, β -Santalene, Junipene, α -Selinene, (+) Longicyclene, τ -Muurolene, Cuparene, β -Bisabolol, Longipinene, (+)-Cyclosativene and limonene. The other wild species *P. cattleianum* formed a separate group which is mainly due to the presence of (Z)-valerenyl acetate, 2,6-Dimethyl-1,4-dihydropyridine-3,5-dicarbonitrile, 4-Isopropyl-1,6-dimethyl-1,2,3,4, 4a,7 hexahydronaphthalene, cadina-1,4-diene, 4-Isopropyl-1,6-dimethyl-1,2,3,4,4a,7 hexahydronaphthalene, τ -cadinene, α -Ferulene, that imparts the unique flavour. The significantly higher concentration of esters, when compared to other accessions, which gives fruity flavor can be incorporated to progenies for crop improvement.

As regards to the cultivated varieties, Benzenepropyl acetate, α -Bisabolene, Allo-Aromadendrene, trans-Ocimene, (-)- β -Cadinene, (Z)-cis- α -bergamotene, cis-Ocimene, β -Bisabolene, Trans- τ -bisabolene, (+)- β -Funebrene, γ -curcumin, τ -Cadinene compounds were found to be predominant in the red fleshed varieties which form a separate group. Similarly due to the presence of Cedr-8-en-13-ol, Nerolidol, 3-Phenylpropyl isobutyrate, 3-Pentyl-1-cyclopentene, α -Bisabolene, Viridiflorol, α -Cubebene, α -Humulene, white fleshed fruits formed a separate group. This indicates that, the aroma components are specific to white and red fleshed varieties and was found to be common in parents and progenies of the respective group.

In a nut shell, the specific aroma compound (Z)-valerenyl acetate of *P. cattleianum*, limonene of *P. chinensis* and limonene and α -Cedrene of *P. guineense* could be exploited in the crop improvement programme.

VI. SUMMARY AND CONCLUSION

Guava (*Psidium guajava* L., $2n = 22$) belongs to the family Myrtaceae, is one of the most important commercial fruit crops of India. The genus *Psidium* includes about 150 species among which only twenty produce edible fruits and is widely distributed all over the equatorial regions of tropical and subtropical climates. Commercial cultivation of guava is common in India, Brazil, Mexico, Florida, Hawaii, California etc.

P. guajava has got adequate genetic variation which is required for any crop improvement programmes. Other *Psidium* species including *P. cattleianum* Sabine, *P. friedrichsthalianum* (O. Berg) Nied., *P. guineense* Sw., *P. montanum* Sw. and *P. sartorianum* (O. Berg) Nied (Pathak and Ojha, 1993) reported to have different utility and are fairly well distributed and thus can be used for crop improvement. These wild species are reported to have resistance or tolerance towards many biotic and abiotic stresses (Landrum *et al.*, 1995 and Edward and Shankar, 1964, Singh *et al.*, 1977).

In order to make use of different genotypes for effective crop improvement programme, it is crucial to know their phenological stages from which the growing behavior will be understood and the chromosome study is important for forecasting compatibility. In order to observe the transfer of gene, aroma can be used as a marker, as transfer of any trait may lead to change in aroma compound of the hybrid. So, as a primary study aroma profiling is important to serve this purpose.

Hence, the present study deals with the phenology of wild species and its crossability with *P. guajava* cultivars, cytological studies and morphological traits, biochemical analysis and volatile aroma profiling of cultivars and wild species of guava that aims at crop improvement. Keeping this information as baseline, an investigation entitled 'Studies on the utilization of wild species of guava (*Psidium guajava* L.) for crop improvement' was carried out.

- A synchronization of peak flowering, duration of flowering as well as anther dehiscence was recorded between Arka Kiran and wild species like *P. guineense* and *P. chinensis*, which is important to carry out effective hybridization work.
- *P. chinensis* was found to be a good combiner with the cultivated species and Arka Rashmi was found to be the best parent compatible with *P. chinensis*.
- In general, *P. chinensis* was found to be a better parent compared to *P. guineense* with regards to per cent fruit set, per cent fruit harvest, number of seeds obtained, and per cent germination.
- Per cent germination of cross combination Arka Mridula X *P. guineense* was found to be better when compared to its cross with *P. chinensis*.
- Cytological studies on cultivars and wild species obtained chromosomes obtained were not in active mitotic metaphase. The chromosome number of the cultivars was approximately $2n=22$. The counting of the chromosome number was not possible due to improper separation of chromosomes. Further standardization of the study with respect to root tip, shoot tip and flower buds is required.
- The wild species were inferior to the cultivars with respect to fruit weight, volume, length, width, thickness of outer pulp in relation to core diameter, core diameter, 100 seed weight, seed number, seed hardness.

- The fruits of wild species were small with hard seeds except *P. chinensis*.
- Interestingly, the peel colour of *P. cattleianum* was red.
- The wild species shows high TSS, titrable acidity, vitamin C, total sugars, reducing sugar and non reducing sugar and was comparable with the cultivars but the total carotenoid and lycopene content of wild species was found to be inferior to the cultivars.
- The hydrocarbon compounds present in wild species were comparable with cultivars whereas the monoterpenoid compounds present in *P. chinensis* was found to be superior compared to cultivars and concentration of limonene was found to be higher.
- Among the sesquiterpenoids, all cultivars and wild species were showing higher concentration of β -Caryophyllene except in *P. guineense* which contains α -Cedrene in higher concentration.
- The alcohol compounds in wild species which contributes to aroma were found to be higher compared to cultivars except in *P. cattleianum*, where the concentration was at par with cultivars and the concentration of aldehyde and ketones in wild species were comparable with cultivars.
- The esters present in *P. cattleianum* were found to be significantly high compared to the other wild species and cultivars whereas the oxo compounds present in wild species were comparable with cultivars.

FUTURE LINE OF WORK

- Widening of genetic base including wild species and allied genera for nematode and wilt complex problem
- Evaluation of the developed F_1 interspecific progenies for abiotic and biotic stress tolerance
- Work on cytological studies of *Psidium* species may be intensified for aneuploidy breeding
- Volatile aroma profiling of selected F_1 interspecific progenies for the use of aroma profiling as a marker

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ANNEXURE I

Weather parameters during the experimental period

Month	Temperature max (°C)	Temperature min (°C)	RH (%)	Rainfall (mm)
Jul-16	29.2	20.6	79.0	174.2
Aug-16	27.0	20.6	80.6	16.5
Sep-16	25.2	21.7	80.5	58
Oct-16	26.0	19.0	58.0	15
Nov-16	27.0	18.0	64.0	9
Dec-16	28.0	17.0	73.0	62
Jan-17	28.6	16.3	72.6	0
Feb-17	28.7	15.1	54.6	0
Mar-17	31.7	17.5	53.1	1.7
Apr-17	30.9	19.2	50.1	1.1
May-17	27.6	20.5	62.1	9.4
June-17	26.8	20.6	70.7	1.9