International Journal of Agriculture Sciences
ISSN: 0975-3710&E-ISSN: 0975-9107, Volume 8, Issue 59, 2016, pp.-3288-3290.
Available online at http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000217

Research Article

ANALYSIS OF MOLECULAR VARIANCE (AMOVA) AND PRINCIPLE COORDINATE ANALYSIS (PCOA) OF GUAVA GERMPLASM

KUMARI SHAILI[1]*, ARUMUGAM NAGARAJA[1], SINGH RAKESH[2], SRIVASTAV MANISH[1], BANOTH SHIVA[1], MITHRA AMITHA CHARU[3] AND GOSWAMI AMIT KUMAR[1]

[1]ICAR - Division of Fruits and Horticultural Technology, Indian Agricultural Research Institute (IARI), New Delhi, 110012
[2]Division of Genomic Resources, ICAR- National Bureau of Plant Genetic Resources, Issapur, Delhi, 110073
[3]ICAR - National Research Centre on Plant Biotechnology, Pusa Campus, New Delhi, Delhi 110012
*Corresponding Author: Email-shailiranchi51@gmail.com

Received: November 23, 2016; Revised: November 29, 2016; Accepted: November 30, 2016; Published: December 06, 2016

Abstract- The present study was carried out at the Division of Fruits and Horticultural Technology ICAR-IARI and at the Division of Genomic Resources, NBPGR, New Delhi during 2013-15. Total 33 genotypes including six species and 28 varieties per genotypes of *Psidium guajava* were used for molecular characterization. DNA markers systems namely, SSR were used for the genetic diversity analysis among guava germplasm. Total of 39 SSR primers were used to characterize the guava genotypes. Out of 39 primers 26 primers were reproducible. The SSR data were used for analysis of molecular variance (AMOVA) and principal coordinate analysis (PCoA). In AMOVA and PCoA three hierarchical levels, individual, population and their group. The analysis was performed by using GenAlEx software. Analysis of molecular variance shows variation at three levels, among individual total variance is 59 %,among population is 1% and within individual its percentage is vary upto 40%. Prinicipal co-ordinate analysis distribute all 33 genotypes of guava in two co-ordinate based on their area of collection, all 33 genotype are divided into six population. This six population are naming based on their area of locality. All population does not show any significant differences among the individual population, which varies only 1%. The result of AMOVA and PCoA are correlated with each other.

Keywords- Characterization, Population, AMOVA, GenAlEx, PCoA

Citation: Kumari Shaili, et al., (2016) Analysis of Molecular Variance (Amova) and Principle Coordinate Analysis (PCoA) of Guava Germplasm. International Journal of Agriculture Sciences, ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 8, Issue 59, pp.-3288-3290.

Academic Editor / Reviewer: Dr B. L. Dudhat, S Sanbagavalli

Introduction

Guava is important fruit crop, which are originated in tropical America. Its belong to the family *Myrtaceae*. It widely grown in tropical and subtropical climate. Guava is one of the hardy fruit crop. It is the richest source of antioxidant and other important nutrients, which are highly beneficial for human health. Apart from its nutritional importance, a wide range of variability exist in India. This variability is important for improvement in further breeding programme. Based on this aspect research was conducted in at the Division of Fruits and Horticultural Technology ICAR-IARI and at the Division of Genomic Resources, NBPGR, New Delhi during 2013-15. To facilitate teaching population genetic analysis GenAlEx 6 software were used at the graduate level, [1]. GenAlEx: means (Genetic Analysis in Excel) which used population genetic analysis by entering the molecular data in Exel sheet. It offers analysis of diploid co-dominant, haploid dominant, binary genetic loci and DNA sequences. Distance-based (AMOVA, PCoA) and Frequency-based (F-statistics, heterozygosity) both type analysis is provided by this software. More than 30 other data formats analyzed by using this software. This software is capable to analyse wide range of population, which provide options for the full spectrum of genetic markers within the Microsoft Excel environment. GenAlEx highly useful for population genetic analysis by students, teachers and researchers. Pair-wise genetic distances as computed using the DISTANCE procedure implemented in GenAlEx 6.5. The same programme was also used to perform a principle coordinate analysis (PCoA), [2]. Principle coordinate analysis of 38 guava accessions from USDA/ ARS, Pacific Basin Tropical Plant Germplasm Resource Center, Hilo, Hawaii and it clustered groups are represented by different colours. The plane of the first three PCoA axes account for a total of 72.1 % of the total variation (first axis=37.8 %, second=19.2 % and third=15.1 %), [3]. Accessions of 12 different region of Pakistan were collected and used it for diversity analysis. By using GenAlEx 6.5 measuring the population at three different levels. The 2D PCA plot successfully grouped the samples based on their phenotypic resemblance and morphological characteristics, [4]. The morphological dendrogram generated from agglomeration hierarchical clustering (AHC) grouped which grouped 132 accessions into 3 major clusters. Different endangered *Mangifera indica* genotypes of Indian Gir forest region were analyzed by RAPD and IISR marker and compare it, [5] .The RAPD and ISSR data were subjected to a hierarchical analysis of molecular variance (AMOVA), as described by [6] using three hierarchical levels; individual, population and their groups.

Material and Method

Guava species and genotypes

Total 33 genotypes including six species and 28 varieties/genotypes of *Psidum guajava* conserved at Division of Fruits and Horticultural Technology, ICAR-IARI, New Delhi, were used for morphological and molecular characterization. Sasri, sasni, Behat coconut, swetha, lalith, A.safeda, L-49, Hafsi red collected from Uttar pradesh, Punjab red, Punjab pink, Snow white collected from Punjab, Pant prabhat, Kaashipur collected from Uttarakhand, Red peel, red type, yellow type,

Pusa srijan, sour type, collected from local area of Delhi. H.safeda, H.surkha, Hissar variant, collected from Haryana. A.kiran and A.amulya collected from Bengaluru. Tamilnadu collection collected from Tamilnadu. Thai, Thai variant 1, Thai variant 2, and Black type are exotic collection and *P. cattleianum*, *P. pumilum*, *P. guinensis*, *P. quadrangularis*, *P. fredrichsthalianum* are conserved from long time used as local collections.

Molecular characterization

Total 39 SSR marker were used for diversity analysis of 33 genotype of guava. To analyse the molecular variance among the population, among individual and within individual was estimated by using GenAlEx software. DNA was isolated by modified method of CTAB followed by DNA purification and DNA quantification which was done in Nanodrop, [7] .To standardize the melting temperature of SSR primer, gradient PCR was set for each primer with selected number of samples of guava. Genomic DNA was diluted to 10 ng/µl for preparing as working stocks. For amplification PCR reaction was set in a thermo-cycler. Amplified PCR product were analyzed on 4% metaphor agarose gel which containing ethidium bromide (10 mg/ml) at a constant voltage of 120V for 4 hours using a horizontal gel electrophoresis system (Biorad, USA).A 100bp DNA ladder (Fermentas, Life Sciences, USA) was used to run alongside the amplified PCR products to determine their approximate band size. Gel pictures were recorded under UV light gel documentation System (Alpha Imager®, USA). Reproducible DNA bands *i.e.*,bands present in both repetitions of individual sample were scored manually. Smears and weak bands were excluded from final data analysis. Band profiles were scored in a binary mode with 1 indicating the presence and 0 indicating the absence of a band. The data matrices thus obtained were compiled in MS-Excel for further analysis.

AMOVA and PCoA analysis

The SSR data were used for analysis the molecular variance in population of guava by using GenAlEx 6.5 software. Pair-wise genetic distances were computed using the DISTANCE procedure implemented in GenAlEx 6.5.

Result and Discussion

For analysis of molecular variance SSR data compiled in MS-Excel . Analysis of molecular variance of 33 genotype of guava is analysed by using GenAlEx 6.5 software. Result indicated the variation among the population is varies 1% in [Fig-1]. Its percentage varies among individual is about 59 %.Within population percentage of variation is 40 %.It shows that highest percentage of variation found among the individual and lowest among populations. Among population variation observed is only 1% which shows that their are very less percentage of variation between the six different population of six different area of collection. AMOVA data directly correlated with PCoA. In PCoA distribution of six different population of guava in two coordinate which shown in [Fig-2]. Distribution of different population is mixed type. Non significant distribution of six populations. It shows that variation among population is very less, it vary only 1%. Existing population is not much variable so that we can't use it for further breeding programme. Among individual variation exist more, in each population. In PCoA this is one of the reason shows that individual of same population shows different distribution in two different coordinate. In first population, which was collected from Bengaluru, total two genotypes exist in this population, Arka amulaya and Arka kiran, which distribute in two different coordinate. This difference observe due to difference in gentic background. Arka amulya is cross of Allahabad safeda and seedless and Arka kiran is a progeny of Kamsari and Purple local. All three genotypes Punjab red, Punjab pink, and Behat coconut, which are collected from Punjab shows three different grouping. Which directly shows that variation among individual is maximum?

Table-1 *Analysis of Molecular variances of 33 genotypes of guava*

Source	df	SS	MS	Est. Var.	%
Among Pops	7	65.502	9.357	0.038	1%
Among Indiv	25	226.558	9.062	3.395	59%
Within Indiv	33	75.000	2.273	2.273	40%
Total	65	367.061	20.692	5.706	100%

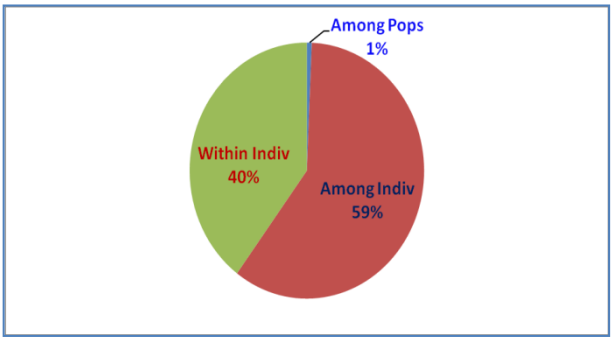

Fig-1 Analysis of molecular variance of 33 genotypes of guava

Table-2 *Principal Coordinates analysis based on molecular data of 33 genotypes of guava*

Axis	1	2	3
%	17.31	10.97	7.77
Cum%	17.31	26.28	36.05

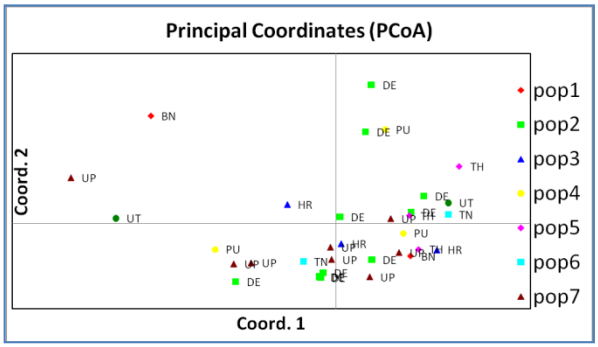

Fig-2 Principal Co-ordinate analysis of six different population of guava

Conclusion

The above result shows that the existing population of guava are very less variable among populations, but the variability is maximum among individual. Analysis of molecular variance is shows non-significant variation among existing population of guava.

Acknowledgement

I express my gratefulness to Dr. Rakesh Singh Principal Scientist, National Bureau of Plant Genetic Resources, New Delhi, for providing me support and technical guidance in all course of my molecular work in his laboratory. I wish to record my sincere innate respect, appreciation and gratitude to my chairmen Dr. A. Nagaraja, and Dr. Manish Srivastav for his valuable suggestions, active cooperation and affectionate encouragement.

Author contribution:

Study conception and Design-Shaili kumari, Dr.A. Nagaraja, and Dr. Manish Srivastav
Technical guidance- Dr.Rakesh Singh
Research carried by-Shaili kumari
Analysis and interpretation of data:-Shaili kumari
Drafting of manuscript: Shaili kumari
Critical revision: Shaili kumari, Shiva banoth, Dr.Amitha charu mithra

Abbreviations:

MS-Excel-Microsoft-Excel
GenAlEx-Genetic analysis in Excel
PCR-Polymerase chain reaction
SSR-Simple sequence repeats

Conflict of Interest: None declared

References:

[1] Peakall R. and Smouse P.E. (2006) *Molecular Ecology Notes,* 6, 288-295.
[2] Peakall R., Smouse P.E. and Huff D.R. (1995) *Molecular Ecology Notes,* l4, 135–147.
[3] Sitther V., Zhang D., Harris D. L., Yadav A. K., Zee F.T., Meinhardt L. W. and Dhekney S.A. (2014) *Genet Resour Crop Evol.,* 61, 829–839.
[4] Mehmooda A., Jaskania M.J., Khana I.A., Ahmada S., Ahmadb R., Luoc S. and Ahmad N.M. (2014) *Scientia Horticulturae,* 172, 221–232.
[5] Gajera H. P., Tomar R. S., Patel S.V., Viradia R.R. and Golakiya B. A. (2011) *J. Plant Biochem. Biotechnol,* 20(2), 217–223.
[6] Excoffier L., Smouse P.E. and Quattro J.M. (1992) *Genetics,* 131,479–491.
[7] Doyle J.J. and Doyle J.L. (1990) *Focus,* 12, 13–15.