International Journal of Innovative Biosciences Research 5(1):1-14, Jan.-Mar., 2017

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Molecular and Morphological Characterization of Pakistani Guava Germplasms

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ABSTRACT

The study was conducted to characterize genetic and morphological characters of eight guava genotypes (Rang wala gola, Saddabahar, Saddabahar barhi surahi, Larrkana, Kohati, Special amrod, Moti surahi wala and Amrod barhi surahi) at National Agricultural Research Centre, Islamabad during 2016. Completely randomized design (CRD) was followed with three replications. In order to study the genetic diversity in these varieties, the randomly amplified polymorphic DNA (RAPD) technique was performed for molecular characterization. For morphological characterization, the morphological descriptors were used. The clustering tree was produced through the results of the seven primers and morphological descriptors through which the genetic and morphological diversity was observed. Through the results produced from the molecular markers and morphological parameters we have found the variation in the genetical make up and physiological appearance in these eight varieties of guava. The clustering tree of similarity among the eight varieties of guava produced through the results of molecular markers and morphological descriptors have difference results in each clustering tree of genetical data and morphological data. The only genetical data could be followed for further researches because the genetical data is more authenticated then the morphological data. There are many possible reasons that the morphological data showing difference from the genetic data for example, the rain-fed conditions, sun light availability to leaves of tree, nutritional problems, competition of plant with weeds, disease problems and etc.

Keywords: Morphological; Genetic; Molecular Marker; Randomly Amplified Polymorphic DNA(RAPD); Polymerase Chain Reaction; Clustering Tree of Similarities; Guava Genotypes; Rain-fed Conditions

INTRODUCTION

The guava *(Psidium guajava)* is a member of the *Myrtaceae* family with (2n=22) consists of fruiting trees and shrubs which has more than 150 species Jaiswal and Jaiswal (6), of which 59 are present in Brazil and 10 are present in Espírito Santo. Guava is being grown all over the sub-tropical and tropical world due to its high dietary value and good flavor. Guava is an important and one of the most nutritious fruit plant of tropical and subtropical regions. Guava fruit contains high amounts of Vitamins A, B1 (Thiamin), B2 (Riboflavin) and C. It is a rich source of vitamin C (Ascorbic acid). The vitamin C contents of guava fruit are four times higher than those of citrus. The leaves of guava have been used for curing diarrhea and dysentery. But unfortunately, in Pakistan people utilize guava just for taste, mostly don't know its nutritional value they eat it because it has a good flavor.

There are many factors affecting the yield of the guava fruit in the regions of Pakistan, the quality and productivity are generally limited by soil salinity and fertility, amongst other abiotic (drought, frost) and

biotic (nematodes, insect pests and diseases) factors. The major factors affecting the quality and productivity of guava in Pakistan presently are the fruit flies which cause the major yield loss in guava crop and its biological control is difficult. Guava breeding could help in enhancing the crop productivity, fruit quality and also control the biotic and abiotic factors, but the first step includes the characterization of the genetic variability in the germlines propagated, in order to detect those trees that can be employed as parents for crop improvement. Although broad phenotypic and productive variability has been found in most orchards from where the germplasm of different guava varieties is collected. The increase in consumption both as a table fruit and natural juices is a worldwide trend; supplementary guava is getting popular over care, health and aesthetics worldwide in the recent years.

Kidaha et al. (7) has studied the morphological characterization of guava landraces from Western and Coastal landraces was done using 13 qualitative and 2 quantitative descriptors. Root descriptors between the landraces in the two regions did not show differences while the leaf, fruit, branching of stem showed variations. Leaf shape varied, being oblong, trapezoidal, elliptical and ovate. Branching habits were either axial, erect or irregular

Randomly amplified polymorphic DNA (RAPD) is a technique which is based on the polymerase chain reaction (PCR) markers, requiring only tiny amount of genomic DNA and does not require expensive material as in molecular technique like radioactive material Malabadi *et al*. (8). There are many plants reported in which the successful application of RAPD technique is used to assess the genetic diversity of different plants. The RAPD markers in particular, have been successfully used to determine genetic diversity among species in tropical and subtropical forest plants Akbar *et al*. (1); Elmeer and Almalki (4); Enjalbert *et al*. (5) and Parkash and Staden (9). Molecular markers are the pre-breeding and advanced tool for the successful assessing of the characterization and evaluation of genetic diversity among different plant species and population Bakhat *et al*. (2). The popularity of PCR is primarily due to its apparent simplicity and high probability of success. Unfortunately, because of the need for DNA sequence information, PCR assays are limited in their application. The discovery that PCR with random primers can be used to amplify a set of randomly distributed loci in any genome facilitated the development of genetic markers for a variety of purposes. The simplicity and applicability of the RAPD technique have captivated many scientist's interests. Perhaps the main reason for the success of RAPD analysis is the gain of a large number of genetic markers that require small amounts of DNA without the requirement for cloning, sequencing or any other form of the molecular characterization of the genome. Keeping all these factors in view, a study was designed on genetic and morphological diversity of eight different guava genotypes.

MATERIAL AND METHODS

Plant materials

To conduct the studies which were taken from guava orchard present in the front of National institute of genomics and advanced biotechnology (NIGAB), National Agriculture Research Centre (NARC), Islamabad, Pakistan. The plants with complete juvenility were selected. For the morphological studies three plants of same variety were selected and average of these three plants were used as the final reading. The names of varieties and their abbreviations are Rang wala gola (RWGola), Saddabahar (SBahar), Saddabahar barhi surahi (SBBSurahi), Larrkana (Larrkana), Kohati (Kohati), Special amrod (SAmrod), Moti surahi wala (MSWala), Amrod barhi surahi (ABSurahi).

DNA Extraction and Quantification

To conduct DNA extraction, the fresh epical meristem from eight varieties was used. To extract DNA, the modified CTAB method described by Doyle and Doyle (3) was used. The DNA extraction was done with great care to minimize the error chances. The mercaptoethanol was used as a replacement of liquid nitrogen. The pellets of DNA were dissolved into 100 µl of deionized water in each sample respectively. The samples were incubated at 4^{o}C for at least 2 hours so the DNA pellets were completely dissolved into the deionized water. The samples were stored at 4^{o}C until the PCR is started. The DNA was quantified through the BioSpec-nano instrument (Table No.3) and the dilutions were prepared for further PCR processes (Table No.4).

Polymerase chain reaction (PCR)

In order to comportment through polymerase chain reaction (PCR) the 20 µl of reaction volume was used in which the 1.5 µl of template DNA and 18.5 µl of master mixed was used. To make master mix following reagents were used 10XPCR buffer, $MgCl_2$, dNTP mix, Primer, Taq Polymerase of Fermentase. The reaction volume was set in the PCR try and the PCR amplification was carried out in an automated Applied Bio-systems Thermal Cycler (variety 96 well) at 94°C for 4 minutes, followed by 45 cycles each consisting of three steps; one step of denaturation at 94°C for 40 seconds, one step of annealing according to temperature of primers (Table No.5) for 40 seconds and an extension step for 1 minute at 72°C. The last step was followed by a final extension of 10 minutes at 72°C. Amplified products were electrophoresed with 6X loading dye on 1.5% agarose gel stained with Ethidium Bromide and subsequently visualized using the Gel Documentation System.

RESULTS AND DISCUSSIONS

Results of Morphological parameters of guava plant

Different morphological parameters were studied in eight varieties of guava *(Psidium guajava L.)* during the research conducted. The studied parameters are qualitative and were collected through visualization and observation of eye. All the descriptors were collected and studied by taking the descriptors following the procedure given by Kidaha *et al.* (7) publication as a standard. The comparison table (Table No.5) was produced and the scoring of parameters was made to study and understand the variation or similarities easily. Another table (Table no.6) was produced and scoring of morphological data was taken with respect to the comparison table. For example, the presence of pubescence of the epical leaves, 1 number was given to those varieties which have no pubescence in their epical leaves and the 2 number was given to those varieties which have pubescence present in their apical leaves. Therefore, all the eight varieties have pubescence in their apical leaves.

Molecular characterization of guava genotypes

DNA Analysis of eight guava varieties by 7 RAPD primers

Seven RAPD primers were used for polymorphism in *(Psidium guajava L.)* initially. Seven primers (OBP1, OPA2, OPA4, OPA5, OPA7, OPA9 and OPA19) were chosen for the RAPD PCR amplifications. Out of these 4 primers have given the polymorphic bands. All these 4 primers showed different bands at different sizes of bp. Others does not show any bands there are many possible reasons that bands are not shown. The size of reproduce able and score able bands (Table No.7) ranged differently in all primers and showed difference in 8 varieties of guava *(Psidium guajava L.)* species. These results indicated that RAPD marker system used in this study revealed a little range of genomic DNA diversity in guava *(Psidium guajava L.)* plant and its related varieties. Little variation is due to less number of plants or varieties available.

In order to score the gel picture produced through the running of PCR product on the 1.5% agarose gel of different primers the producible and score able bands of different base pair sizes were scored as 1 as presence of the band and the 0 as the absence of the respective band at respective base pair size in the gel picture.

DNA markers data based clustering of Guava genotypes

In order to probe into the genetic diversity between different eight varieties of guava (*Psidium guajava L.)* a reconstruction was carried out. The sequences and scoring of molecular marker were edited using NTedit and a neighbor joining tree was constructed by using NTSYSpc 2.1 software. The clustering tree of similarity (Fig No. 6) showed that there was a clear image of the genetic diversity present in eight varieties of guava. The tree showed differentiation into different clades. The Kohati is seems sister to SBBSurahi and MSWala is sister to SAmrod. Through this tree we have found that there is no major genetic diversity in between the RWGola and Larrkana varieties of guava. However, the ABSurahi seems to be the progenitors according the results of these 7 primers used in these eight varieties of the guava.

Morphological parameters based clustering of Guava genotypes

While studding the morphological diversity between different eight varieties a clustering tree of similarity was produced and a reconstruction was carried out. The qualitative readings were edited using NTedit and

a neighbor joining tree was constructed by using NTSYSpc 2.1 software. The figure produced (Fig No. 7) showed the clear morphological diversity present in eight varieties of guava. The tree showed differentiation into different clades. In this dendogram of morphological similarities, there were two main groups G-1 and G-2. The group G-1 leads toward two further groups G-1-1 and G-1-2, the group G-1-1 leads to two further groups G-1-1-1 and G-1-1-2 with respective varieties named as RWGola and SAmrod. The group G-1-2 leads to two groups G-1-2-1 and G-1-2-2 in which the group G-1-2-1 leads to two varieties with no diversity named as SBahar and Larkana, while the group G-1-2-2 leads to the single variety SBBSurahi. The group G-2 splits into two groups G-2-1 and G-2-2, the group G-2-1 leads to G-2-1-1 and G-2-1-2 with Kohati and MSWala varieties of guava respectively. The group G-2-2 leads to the ABSurahi variety.

In both dendograms there were no similar results about the diversity present in the both results. The morphological study of parameters showed different tree of similarity in the eight varieties and the results of molecular markers showed different results. In the both results the results of molecular markers were more authentic compared to the morphological results. There were many possibilities which can lead to the difference in the results of both dendograms. And the growth of the plant of same varieties at the same field could be different in many ways. For example, the effect of nutrient on a plant, the effect of sun light available to the plant, the effect of shade on the leaves of one plant to another plant, the water availability and water stress, humidity, effect of pathogens and insects on the plant and many more environmental factors can affect the change in the growing habit of plant which results in the difference in the growth of the plant of same variety at the same place. The molecular markers were more reliable because it takes generation to change the genetic makeup of the plant or any organism. Through these results, we can say that the morphological parameters were not so confirm compared to the genetic parameters that we can be able to describe any diversity or similarity in the eight varieties of guava.

CUNCLUSIONS

The conclusions have been extracted were, in order to full fill the requirements of the remarkable breeding programs the massive knowledge about the progenies and germplasm characterization are required. That characterization was done through the molecular markers and physiological descriptors in the following research which has given the authenticated results through the molecular markers in comparison to the physiological descriptors. The selection of the plants for the breeding of the guava improvement could be easier in these eight varieties of Pakistani guava.

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Table 2: Actual DNA concentrations in the samples extracted.

Sample No.	DNA concentration ηg/µl	Sample No.	DNA concentration ηg/µl
1	1731.26	**5**	1877.30
2	2420.31	**6**	348.120
3	3254.67	**7**	3708.32
4	2268.51	**8**	1574.39

Table 3: DNA concentrations used for the PCR.

Sample No.	Approximate Dilutions of DNA for 50 ηg/µl	Approximate Dilutions of DNA for 100 ηg/µl
1	1:34.6	1:17.3
2	1:48.4	1:24.2
3	1:65.1	1:32.5
4	1:45.3	1:22.7
5	1:37.5	1:18.8
6	1:7	1:3.5
7	1:74.1	1:37.1
8	1:31.5	1:15.7

Table 4: Primers used for PCR.

Sr. No.	Name of primer	Sequence of primer	Tm
1	OBP1	GTTTCGCTCC	34.3
2	OPA2	TGCCGAGCTG	42
3	OPA4	AATCGGGCTG	36
4	OPA5	AGGGGTCTTG	33.5
5	OPA7	GAAACGGGTG	34.1
6	OPA9	GGGTAACGCC	38.8
7	OPA19	CAAACGTCGG	35.1

Table 5: Comparison of different parameters

• Color of apical leaves	1. Yellowish green with brown margins	2. Light green with brown margins	3. Yellowish green with light brown margins	4. Light green with light brown margins	5. Greenish brown
• Pubescence on apical leaves	1. Absent	2. Present			
• Leaf petiole color	1. Yellowish green	2. Light green	3. Green	4. Reddish green	
• Color of leaf vein	1. Yellowish green	2. Light green	3. Green		
• Mature leaf color	1. Light green	2. Green	3. Dark green	4. Maroon green	
• Leaf shape	1. Ovate	2. Obvate	3. Oblong	4. Round	
• Leaf shape form base of leaf	1. Obtuse	2. Round	3. Cordate		
• Leaf shape from tip of leaf	1. Obtuse	2. Round	3. Acute	4. Apiculate	
• Leaf twisting	1. Absent	2. Little present	3. Present		
• Branch color	1. Light grey	2. Brownish grey	3. Dark grey		
• Branching habit	1. Irregular	2. Semi erect	3. Erect		

All the descriptors for morphological data are taken and studied by Kidaha *et al* (2015). Some parameters are added more to understand variation in guava varieties, like brownish gray and semi erect habit of plant branch color and branching habit respectively.

Table 6: Morphological Scored Data Table

Morphological parameters	Name of Varieties							
	RWGola	SBahar	SBBSurahi	Larkana	Kohati	SAmrod	MSWala	ABSurahi
1) Color of apical leaves	4	1	3	3	3	5	2	2
2) Pubescence on apical leaves	2	2	2	2	2	2	2	2
3) Leaf petiole color	1	3	3	3	4	4	3	1
4) Color of leaf vein	1	2	2	2	2	2	1	1
5) Mature leaf color	4	4	4	4	2	4	1	3
6) Leaf shape	2	3	1	1	3	1	3	1
7) Leaf shape form base of leaves	1	2	2	2	3	1	1	2
8) Leaf shape from tip of leaves	1	1	2	1	1	1	1	2
9) Leaf twisting	3	2	1	2	1	3	2	1
10) Branch color	2	2	1	2	3	3	3	3
11) Branching habit	1	1	1	1	3	1	3	3

Table 7: Bands scored data at different bp of different primers

Name of Varieties	**OBP 1 A**	**OBP 1 B**	**OBP 1 C**	**OPA 2 A**	**OPA 2 B**	**OPA 2 C**	**OPA 2 D**	**OPA 2 E**	**OPA 4 A**	**OPA 4 B**	**OPA 5 A**
1.Rang wala gola	1	1	0	1	0	0	0	0	0	0	1
2.Saddabahar	1	1	1	1	1	0	0	0	0	0	1
3.Saddabahar barhi surahi	1	1	0	1	1	1	1	0	0	1	0
4.Larrkana	1	1	0	1	0	0	0	0	0	1	1
5.Kohati	1	1	0	1	1	1	1	1	0	0	0
6.Special amrod	1	1	1	1	0	1	1	0	1	1	0
7.Moti surahi wala	1	1	0	1	0	1	1	1	1	1	0
8.Amrod barhi surahi	1	0	1	0	0	0	0	0	1	1	0

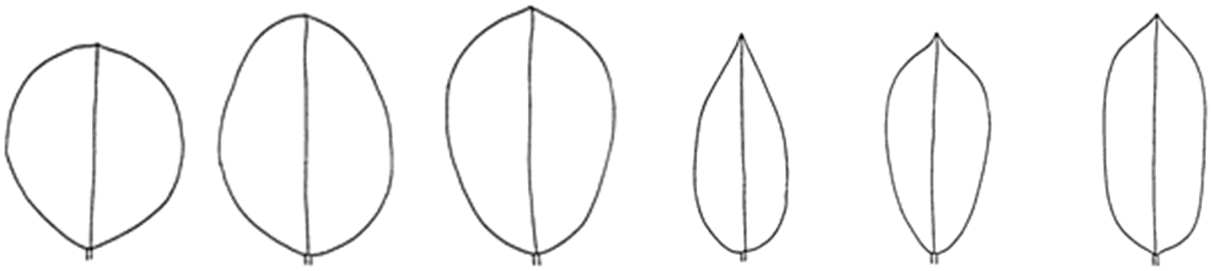

Fig. 1: Leaf shapes

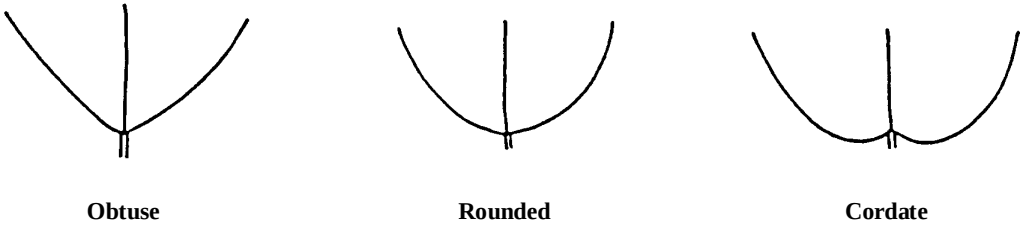

Fig. 2: Fully developed leaf shapes from base

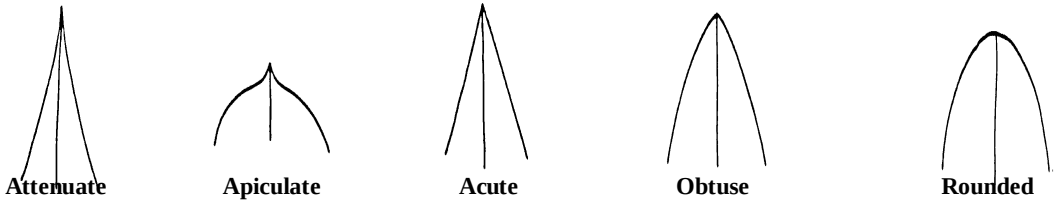

Fig. 3: Fully developed leaf shape of leaf tips

All the descriptor pictures showing different shapes for morphological parameters are taken and studied by Kidaha *et al* (2015).

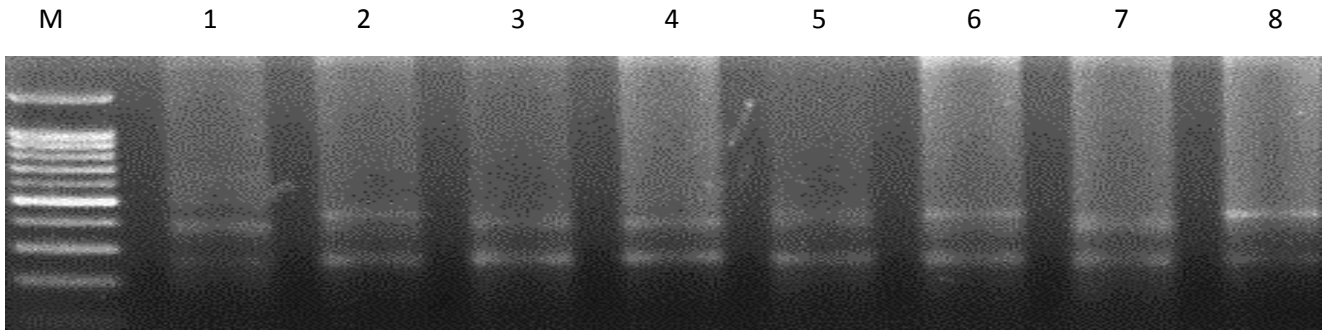

Fig. 4: Gel picture of OBP 1 Primer with 100bp Ladder

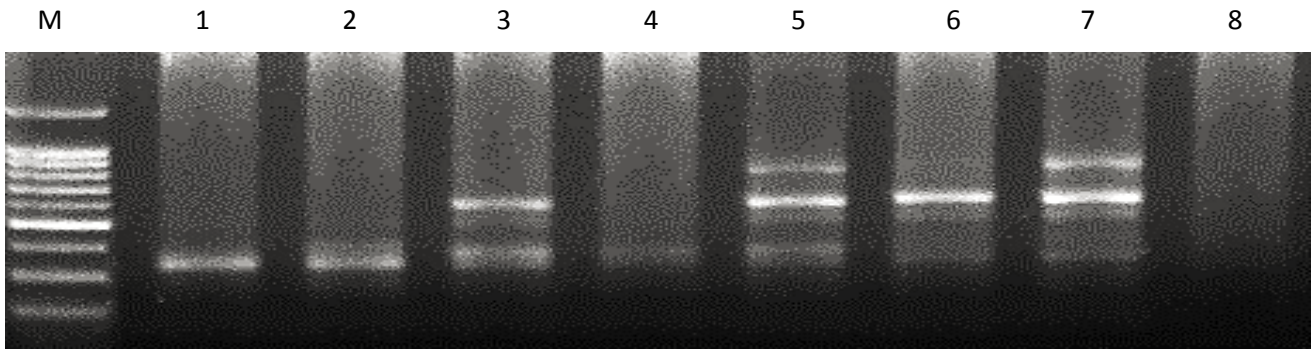

Fig. 5: Gel picture of OPA 2 Primer with 100bp Ladder

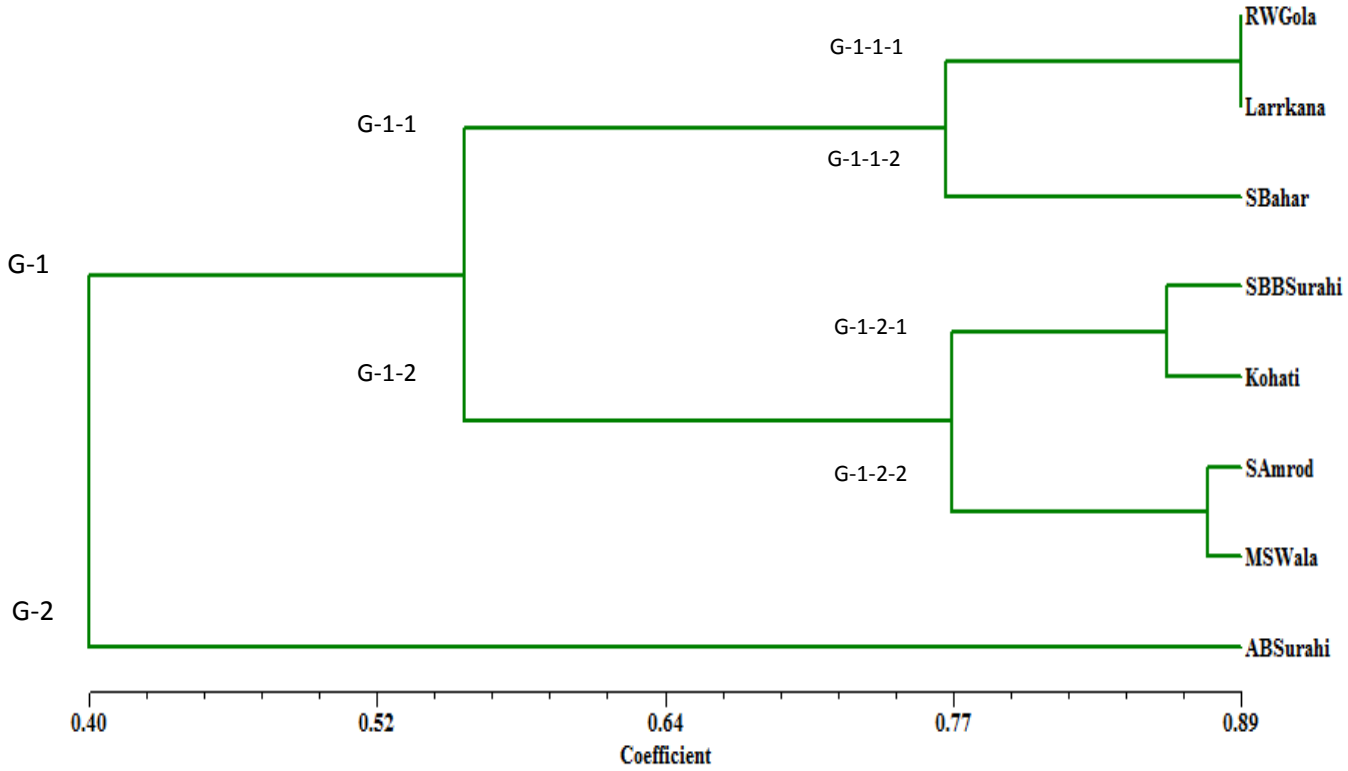

Fig. 6: DNA markers data based clustering of Guava genotypes

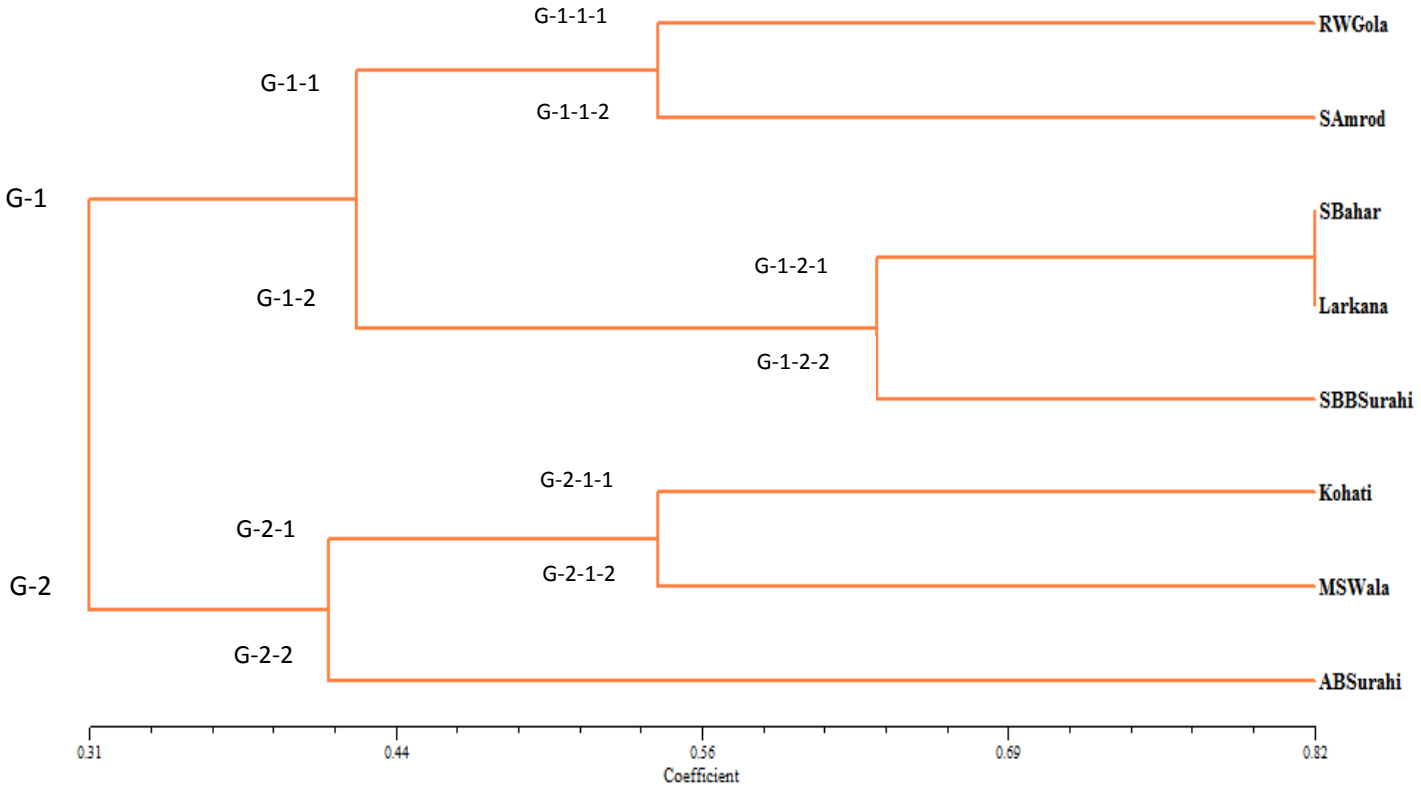

Fig. 7: Morphological parameters based clustering of Guava genotypes