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OBSERVATION OF GENETIC VARIATION AMONG *Psidium guajava.L* VARIETIES USING RAPD TECHNOLOGY

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ABSTRACT

The aim of the study to Observation of genetic variation among *Psidium guajava.L* varieties using rapid technology. The main aim of this work was to determine the genetic variation in different varieties of same related species of Guava genetic variation is also called as genetic diversity. Genetic diversity refers to the variation of genes within species. *Psidium guavajava.*L (guava) seeds obtain seedlings whose first pair of leaves were used for the extraction of genomic DNA and was performed by the CTAB analysed to know the pure quality of DNA. RAPD analysis had been successfully used in mapping and finger printing applications. All the primers cannot separate all the varieties of Guava but Genetic variation in the experiment was seen as certain primers could separate.

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INTRODUCTION

Guavas are evergreen shallow rooted shrubs or small trees to 33 feet, with spreading branches. The bark is smooth, mottled green or reddish brown and peels off in their flakes to reveal the attractive 'bony' aspect of its trunk.

Foliage:

Guava leaves are opposite, short petiole, oval or oblong – elliptic, somewhat irregular in outline 2 – 6 inches long and 1 – 2 inches wide. The dull-green stiff but leathery leaves have pronounced veins, and are slightly downy on the underside, crushed leaves are aeromatic.

Related species of Guava are

Techniques to detect Genetic variation:

In principle, any method that enables genetic differences to be detected between individuals can be applied to population level survey to the genetic variation.

Plant DNA finger printing:

DNA finger printing can be of use to plant breeders to produce desirable new traits. The DNA test can also be used to identify and transferred into the crops. As well it can become a tool to simplify the most traditional methods of selective breeding, by identifying what are known as markers. Here Molecular Markers are using SSRs (Simple Sequence Repeats) also known as Micro satellites.

Micro satellites are tandem repeats of sequence units generally less than 5Bp in length of TG_{cn} (or) $(AAT)_n$. 1 to 6 nucleotides in length which show a high degree of polymorphism. The main advantage of using SSRs for finger printing is that small amounts of DNA are required compared to the restriction fragment length polymorphism (RFLP) method. Further assays involving SSRs are more rebust than random amplified polymorphic DNA (RAPD). MARTIN – C, (2002)

Materials and methods:

Plants:- In the present sample seeds are shown to obtain seedlings whose first pair of leaves were used for the extraction of Genomic DNA. Observation was main between the four varieties of Guava were Red Indian, Ruby X, Sweet white Indonesian and white Indian type.

1) (Varieties differ widely in flavour and seed liners. Actual seed counts have ranged from 112 to 535. Indian journal of clinical practice vol -10. March 2000).

Experimental design:

In the present experiment, first extraction of DNA from 4 varieties of Guava is done with CTAB method. Then the extracted DNA is made to run on agarose gel and analysed under UV trans illuminator. Then the extracted DNA is amplified with the process of PCR with 20 primers that is 220 Reactions. Then again these results are seen in agarose gel and seen under UV trans illuminator attached with a computer for visualizing the polymorphisms among the varieties with 30 primers. The PCR is a powerful method for fast invitro enzymatic amplification of specific DNA sequences.(ZHOU-CHUNLING -2002)

APPENDIX

Genomic DNA isolation:

* CTAB STOCK: Log acetyl tri methyl ammonium bromide is dissolved in 100 ml of double distilled water (heat for dissolving completely, autoclave and store at room temperature)

*1M TRIS HCL (pH8.0): 6.057 gm Tris in 50 ml double distilled water. (Autoclave and store at room temperature)

*0.5M NaCl: 14.96 gm of NaCl in 50 ml double distilled water (Autoclave and store at room temperature)

*0.5M EDTA (pH8.0): 0.7306 gm of EDTA in 10 ml double distilled water

*Extraction buffer – CTAB Buffer:

*100mM Tris HCl, pH 8.0 – 0.5 ml from 1M stock.

*100M EDTA, pH 8.0 – 0.1 ml from 0.5m stock.

*1.4 NaCl – 1.4 ml from 5m stock

*1% NaCl – 0.5 ml from 10% stock

*0.2% , mercaptoethanol – 0.01 ml

*(Mercaptoethanol to be added just before use) make up the volume to 50 ml. 3M Sodium acetate (pH 5.2): dissolve 40.81 gm sodium acetate: make the volume to 100 ml. Autoclave and store at room temperature.

*1xTE: 10mM Tris HCl, pH 8.0 - 10µl from 1M stock.

*1mM EDTA, oH 8.0 - 2µl from 0.5m stock.

* Make up the volume to 1ml. (PCR Protocol, Jhon.M.S.Bartlett,)

Amplifications of DNA

Ingredients	**Stock solution**	**Volume to be taken**
Template DNA	50-200mg	2.5µl
DNTPS	2.5	1.0µl
PCR Buffer	10x	2.5 µl
Primers	5-10 Pico moles	1.0 µl
Taq DNA polymerase	1 unit	0.33 µl
Sterile water	1 unit	17.67 µl
Total	-	25.00 µl

PCR buffer (10x) 10mM Tris HC1 P^H: 8.8

500mM KCL

15Mm $Mgcl_2$

0.1% Gelatin

0.05% Tween-20 And 0.05% NP 40

Primers used and their sequence in 5' – 3' directions:-

Prime Number	**Sequence in 5' – 3' Direction**
1	CCGTCGTAG
2	GGAAACCCT
3	TGGCGCACAC
4	GGCACGCGTT
5	CCCGAAGCGA
6	GTGGCTTGGA
7	GTAAACCGCC

8	GTACGGACC
9	GGGCGACTAC
10	TTCCCTCCCA
11	GTGCGCAATG
12	CCTGTACCGA
13	CCTACCGTGG
14	AAGTGCGACC
15	CCTCCTTCTC
16	CCCGGATGGT
17	TCGCATCCAG
18	CTGGCGTGTC
19	ACACCGATGG
20	CTTCTCGGAC

AGAROSE GEL ELECTROPHORESIS:

Buffer:

Buffer	Component	10x
Tris acetic acid, EDTA, TAE Buffer (1x)	40mM Tris acetate pH(7.6) 1mM sod.EDTA	48.4 gm Tris base, 11.4 ml acetic acid, 20 ml of 0.5M sod. EDTA.$2H_2O$ (pH 8.0)
Tris Borate EDTA TBE Buffer (0.5x)	89mM Tris borate (pH 8.3) 1mM sod.EDTA	108 gm. Tris base 55 gm. Boric acid 9.3 gm. sod.EDTA.$2H_2O$
Tris phosphate EDTA TPE Buffer (1x)	89mM Tris phosphate 2mM EDTA	108 gm. Tris base 15.5 ml 85% H_3PO_4, 40 ml 0.5 M sod. EDTA (pH 8.0)

Stock solution stored at 4°C

GEL LOADING BUFFER:

Buffer	6x
SBX	4% (w/v) Sucrose .025% (w/v) Bromophenol blue,0.25% (w/v) xylene cyanol
FBX	15% (w/v) ficoll 400, 0.25%(w/v) Bromophenol blue 0.25% (w/v) xylene Cyanol
GBX	30% (w/v) glycerol, 0.25% (w/v) Bromophenol blue, .025% (w/v) xylene cyanol

SBX and GBX stored at 4°C, FBX stored at room temperature

Stain	Molecular weight
Acridine Orange	301.8
Ethidium Bromide	394.3

DYE USED IN LOADING BUFFER:

Dye	Molecular weight
Bromophenol	670
Bromophenol	698
Xylene cyanol	554.6

RESULT AND DISCUSSION:

Primer OPA_2 and Primer OPA_{10}:

Primer OPA_2:

No amplification was observed in all the varieties. Primer A_2 cannot be used as differential marker.

Primer OPA_{10}:

Primer A_{10}, in second variety 4 bands is similar they were monomorphic but variety 1, 3, 4 no amplification occurs. Primer OPA_{10} cannot be used as differential marker.

Primer OP_{13} and Primer OPW_{19}:

Primer OP_{13}:

Two different bands are observed in the variety 1 – they showing polymorphism. No amplification was observed in variety two, is they were not having specific sequence variety 3 and 4 similar bands were observed they showing monomorphic pattern.

Primer OPW_{19}:

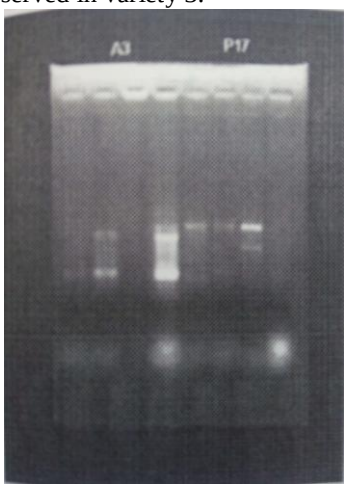
No amplification was observed in variety one. Variety 2 – two thin bands was observed. Variety 3 and 4 no amplification was observed.

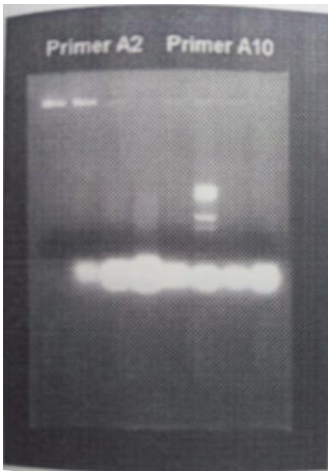
Primer P_{17} and Primer A_3:
Primer P_{17}:

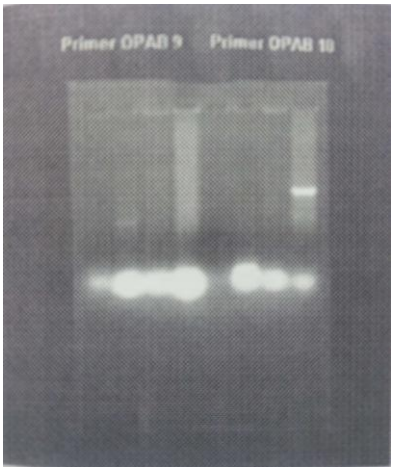
Three different bands were observed in varieties 1, 2 and 3 – they showing polymorphism. Variety 4 – no amplification was observed.

Primer A_3:

All bands are similar in variety 1, 2 and 4 they showing monomorphism. No amplification was observed in variety 3.







Primer $OPAB_{11}$ & primer $OPAB_{12}$

Primer $OPAB_{11}$:

Three different bands were observed in variety in 1,2, & 3 –they were showing polymorphic.

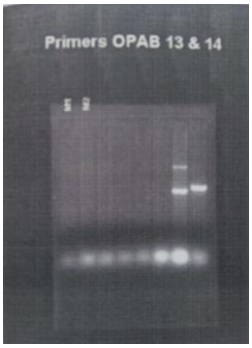
Primer $OPAB_{12}$

Two similar bands were observed in variety 1, &3. They showing monomorphism.Different bands were observed in variety 2 – they showing polymorphic pattern. No amplification in variety 4.

Primer $OPAB_{13}$ & primer $OPAB_{14}$:
Primer $OPAB_{13}$:

No amplification was observed in all the varieties. Primer $OPAB_{13}$ cannot be used to different marker.

Primer $OPAB_{14}$:



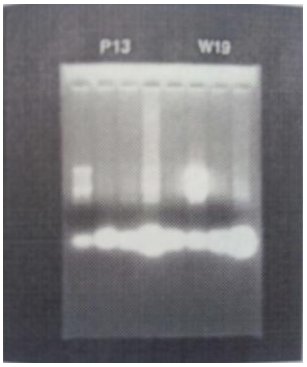
No amplification was observed in variety 1 & 2. Primer $OPAB_{14}$ cannot be used differential marker. Different bands were observed in variety 3&4.They showing polymorphism.

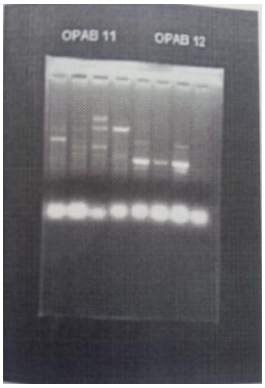
Primer $OBAP_9$ & primer $OPAB_{10}$:
Primer $OPAB_9$:

No amplification was observed in the variety 1, 3 & 4.Primer $OPAB_9$ cannot be used in different marker. Three bands were observed in variety 2.

Primer $OPAB_{10}$:

No amplification was observed in variety 1, 2, & 3.Primer cannot be used in different marker. Bands were observed in variety 4 only bands. No amplification was observed in variety 4.





SUMMARY AND CONCLUSION:

The polymorphic observation was main between the four varieties. Polymorphism between genotypes is due to either a Nucleotide base change that alters the ability of the primer to anneal to the DNA template or an insertion or deletion with in the amplified fragment. Therefore the polymorphism is generally seen as presence or absence of particular RAPD products. This polymorphism can be mapped in the same fashion as other molecular markers, the level of polymorphism detected by RFLPs and RAPD loci are distributed as randomly throughout the genome.(3) KISHIMOTO-S ,etal.)

The PCR is a powerful method for fast invitro enzymatic amplification of specific DNA

sequences. All the primers cannot separate all the varieties of Guava but genetic variation in the experiment was seen as certain primes could separate.

These RAPD markers can help in the variety and identification of a fasters and more accurate than the conventional technique as it can avoid the manual mistakes which are subjected to happen during manual sousing of the fruit characters. These RAPD markers thus can be further used to developing dendro grams, which can be used by plant breeders.

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