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## Research Article

### Molecular characterization of traditional mango germplasm of coastal Andhra Pradesh using RAPD markers

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#### Abstract

A study was conducted at Horticultural research station, Venkataramannagudem for evaluating the variability of mango germplasm to conserve the elite ones and to identify the superior genotypes using molecular markers for future crop improvement. Genetic variation and relationship among 34 traditional mango germplasm were analyzed using Random Amplified Polymorphic DNA (RAPD) markers. Fifteen out of the twenty primers screened were informative and 177 amplified DNA bands with sizes ranging from 100 - 5000 bp were selected as RAPD markers. The number of amplified fragments varied from 3 (OPG 13) to 20 (OPX 04) with an average of 11.8 polymorphic fragments per marker. Specific RAPD markers for some mango germplasm were identified. Unweighted Pair Group Method using Arithmetic means (UPGMA) cluster analysis grouped all the germplasm into two clusters with a genetic similarity coefficient range of 0.67 to 0.88. The accessions within the germplasm i.e., Banganapalli -1, Banganapalli -2 and Banginapalli-3 showed genetic similarity which is in good agreement from a single ancestor with their putative pedigrees. The genotypes Panukula Mamidi and Rajamamidi were closely clustered since these two genotypes are native to the Vizianagaram district. Similarly, the genotypes Nuzividu Tiyya Mamidi and Nuzividu Rasalu occurred in the same cluster indicated that these genotypes also originated from the same geographical area, it can be concluded that they were separated into the cluster based on geographic origin. This study showed clearly that germplasm from coastal Andhra Pradesh unveiled maximum diversity and indicated the potential of RAPD markers for the identification of mango germplasm for breeding purposes.

**Keywords:** Cluster analysis, Dendrogram, Mango, Polymorphic information content and RAPD markers

#### INTRODUCTION

Mango (*Mangifera indica* L.) is an important member of the family Anacardiaceae in order Sapindales and is believed to have originated in the Indo-Burma (Myanmar) region. In India mango ranks first in terms of area with 2.26 million ha, second in respect of production with 21.82 million tonnes and with a productivity of 9.7 million tonnes/ha, while Andhra Pradesh ranks second in terms of area with 0.36 million ha, second in respect to the production of 4.37 million tonnes and with a productivity of 12.05 million tonnes/ha (NHB, 2018). Enormous genetic diversity of mango exists in India, which is the primary center of domestication. Considerable

genetic diversity of this fruit exists in Andhra Pradesh with several named local germplasm and unnamed local landraces. Differentiation of germplasm through morphological features is inefficient and inaccurate. Confusion exists in the nomenclature of mangoes due to different local names for the same variety. This problem is further compounded as mango is highly heterozygous as performance varies with the climate which resulted in a high level of genetic diversity. Many of these complications of a phenotype-based assay can be overcome through direct identification of genotype with DNA- based genetic markers.

Polymerase Chain Reaction (PCR) technology had led to the development of several novel genetic assays based on selective DNA amplification. The protocol is also relatively quick and easy to perform. Because the RAPD technique is an amplification-based assay, only nanogram quantities of DNA are required. One of the strengths of these new assays is that they are more amenable to automation than conventional techniques. It is simple to perform and is preferable to analyze where the genotypes of a large number of individuals are to be determined at a few genetic loci. With this idea, the experiment was undertaken to evaluate genetic variation and relationships of traditional mango germplasm in the coastal Andhra Pradesh by RAPD markers.

## MATERIALS AND METHODS

The present study was conducted to study the performance of the traditional mango germplasm of coastal Andhra Pradesh at Horticultural Research Station, Venkataramannagudem. A well-planned germplasm collection survey based on diversity richness was conducted in coastal Andhra Pradesh which includes Horticultural Research Station and private owned mango orchards. In order to carry out RAPD analysis, leaves from each of the 34 germplasm were collected randomly. Total genomic DNA was extracted by using the modified CTAB (Cetyl Trimethyl Ammonium Bromide) method (Murray and Thompson, 1980). Finally, the DNA samples were stored at - 20°C. The concentration and quality of DNA were estimated by using NanoDrop spectrophotometer at 260 nm and verified by running a sample on 0.8 per cent agarose gel.

DNA amplification was done using fifteen arbitrary decamer primers (Operon Technologies, Inc., Alameda, California, USA OPA-02,03,05,10,18,20, OPB-18, OPG-02,03,10, 11,13 and OPX-04,06,12) adopting the procedure of Williams *et al.* (1990) with some modifications. PCR reactions were performed on each DNA sample in a 25 µl reaction mix containing 50 ng of template DNA, 1 unit of Taq polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India), MgCl<sub>2</sub> - 2.5 mM; dNTPs (Genei) - 0.2 mM; 10 p mole of Random decamer primer (Operon, USA), Buffer (Genei) – 10X and rest amount of sterile deionized water to prepare 25µl reaction mixtures. DNA amplification was performed in an oil-free thermal cycler (Thermal Cycler, Eppendorf). The reaction mix was preheated at 94°C for three minutes followed by 40 cycles of one min denaturation at 92°C one min annealing at 36°C and elongation or extension at 72°C for two minutes. After the last cycle, a final step of ten minutes at 72°C was added to allow complete extension of all amplified fragments. After completion of the cycling programme, reactions were held at 4°C. The amplified products were separated electrophoretically on a 1.4% agarose gel. Medium range molecular weight DNA Ruler (Genei) ladder was electrophoresed alongside the RAPD reactions. Electrophoresis was carried out at 60-90 V for

2-3 hrs and the DNA bands were photographed under UV light by using Major science UVDI gel documentation system.

Since RAPD markers are dominant, it is assumed that each band represented the phenotype at a single allelic locus (Williams *et al.*, 1990). All distinct bands or fragments (RAPD markers) were thereby given identification numbers according to their position on gel and scored visually on the basis of their presence (1) or absence (0), separately for each cultivar and each primer. The scores obtained using all the primers in the RAPD analysis were then pooled to create a single data matrix and used to construct a UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram among germplasm using a computer program, the NTSYS-pc software package version 2.02 (Rohlf, 2000).

## RESULTS AND DISCUSSION

The fifteen primers generated 177 bands with sizes ranged from 100 - 5000 bp (**Table 1**). This was comparable with the results ranging from 200 bp to 3000 bp (Kumar *et al.*, 2001), 190 bp to 2400 bp (Karihaloo *et al.*, 2003), 105 bp to 1500 bp (Rahman *et al.*, 2007), 250 bp to 2500 bp (Anuj *et al.*, 2007), 240 bp to 2100 bp (Ahmad *et al.*, 2008), 300 bp to 3500 bp (Bajpai *et al.*, 2008), 100 bp to 3000bp (Das *et al.*, 2010) and 70 bp to 4749 bp (Gajera *et al.*, 2011) in mango.

The number of amplified fragments varied from 3 (OPG 13) to 20 (OPX 04) with an average of 11.8 polymorphic fragments per primer (**Table 1**). Schnell and Knight (1993), Kumar *et al.* (2001), Xie Jiang Hui *et al.* (2005), Anuj *et al.* (2007), Bajpai *et al.* (2008), Fitmawati and Puwoko, (2010), Gajera *et al.* (2011), Roy and Abhishek (2011) and Abou-Elial *et al.* (2014) reported amplification of 10.90, 13.90, 11.70, 10.53, 12.22, 9.28, 8.90, 10.66 and 16.10 bands per primer, respectively in mango.

The polymorphic information content values (PIC) were calculated for RAPD markers and presented in **Table 1**. The highest PIC was recorded by the RAPD marker OPA 05 (0.97) while, it was found to be the lowest for the marker OPG 13 (0.60). Higher the PIC value more is informative of RAPD marker and hence, the primer OPA 05 was found to be highly informative.

The most important function of a primer is to discriminate as many germplasm as possible, which could be exploited for DNA fingerprinting. The polymorphic fragments present in only one cultivar were considered to be unique fragments (**Table 2**). Out of the total 177 polymorphic fragments, nineteen fragments were unique to particular germplasm viz., Chinna Suvarnakha (OPA 03), Navaneetham (OPA 03), Rajamanu (OPA 10), Elamandala (OPA 10), Baramasi (OPB 18), Banglora-1 (OPG 11), Jehangir (OPA 20), Banganapalli -3 (OPX 04), Kowsuri Pasand (OPX 04), Banganapalli -1(OPX 06) and

Table 1. Details of random primers used for DNA amplification in mango germplasm

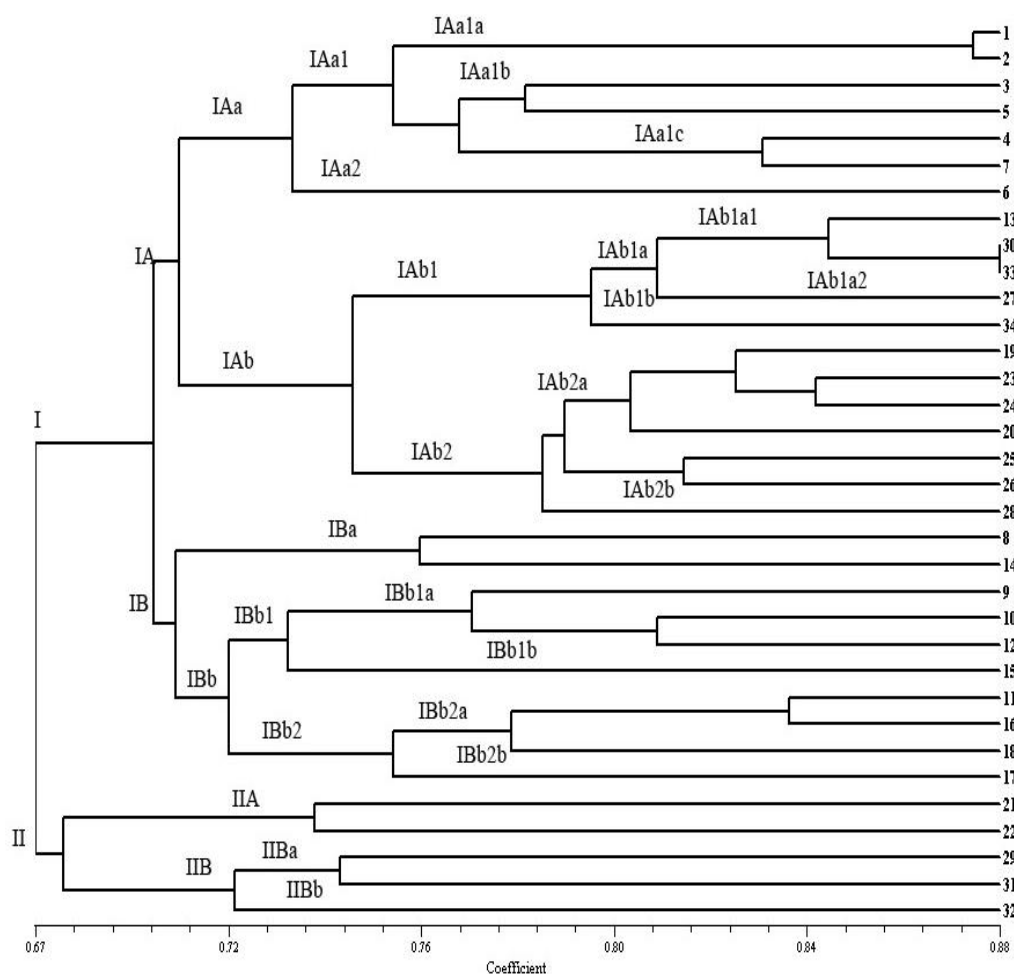
S. No.	Primer code	Total number of alleles	Range of fragment size (bp)	PIC
1	OPA – 02	15	160-1185	0.76
2	OPA – 03	12	120-1150	0.90
3	OPA – 10	19	280-1500	0.82
4	OPA – 18	12	200-1500	0.75
5	OPB – 18	17	200-5000	0.60
6	OPG – 02	12	200-1900	0.79
7	OPG – 11	11	300-1185	0.89
8	OPX – 12	11	250-1500	0.70
9	OPA – 20	10	250-1500	0.73
10	OPG – 10	15	180-2500	0.83
11	OPX – 04	20	180-3000	0.96
12	OPX – 06	9	200-1450	0.93
13	OPG – 13	3	150-400	0.61
14	OPA – 05	6	350-1185	0.97
15	OPG – 03	5	100-900	0.95
<b>Total</b>		<b>177</b>		
<b>Average</b>		<b>11.8</b>		<b>0.81</b>

Table 2. Details of unique RAPD fragments associated with specific mango germplasm

S. No.	Name of the cultivar	Primer revealing unique RAPD	Size of the fragment (bp)
1	Banglora -2	OPA 02	200
2	Chinna Suvarnarekha	OPA 03	500
3	Navaneetham	OPA 03	800
4	Rajamanu	OPA 10	980
5	Nalla Andrews	OPA 10	1230
6	Elamandala	OPA 10	1400
7	Hyder	OPA 18	1050
8	Baramasi	OPB 18	200
9	Banglora - 2	OPB 18	1000
10	Nalla Andrews	OPG 11	700
11	Banglora - 1	OPG 11	730
12	Hyder	OPG 11	1000
13	Jehangir	OPA 20	1300
14	Nuzividu Tiyya Mamidi	OPX 04	180, 380
15	Banganapalli -3	OPX 04	1500
16	Kowsuri Pasand	OPX 04	2500
17	Banganapalli -1	OPX 06	600
18	Nalla Rasalu	OPG 03	200

Nalla Rasalu (OPG 03) while, Banglora -2 (OPA 02 and OPB 18), Nalla Andrews (OPG 11 and OPA 10) and Hyder (OPA 18 and OPG 11) produced each unique fragments with two primers and Nuzividu Tiyya Mamidi (OPX 04) produced two unique fragments with only OPX 04 primer at 180 and 380 bp which could be exploited for DNA fingerprinting of these germplasms by converting RAPD markers into STS (Sequence Tagged Site) markers, which

would be useful for detecting mixes between germplasm (Fernandez *et al.*, 2002 and Chimote *et al.*, 2007). OPA 10, OPG 11, OPX 04 amplifies more unique molecular fingerprints to identify the maximum each three cultivars. No single RAPD primer could distinguish all cultivars independently. These DNA markers have the potential to be used in mango for marker-assisted selection (MAS) and for cultivar identification.



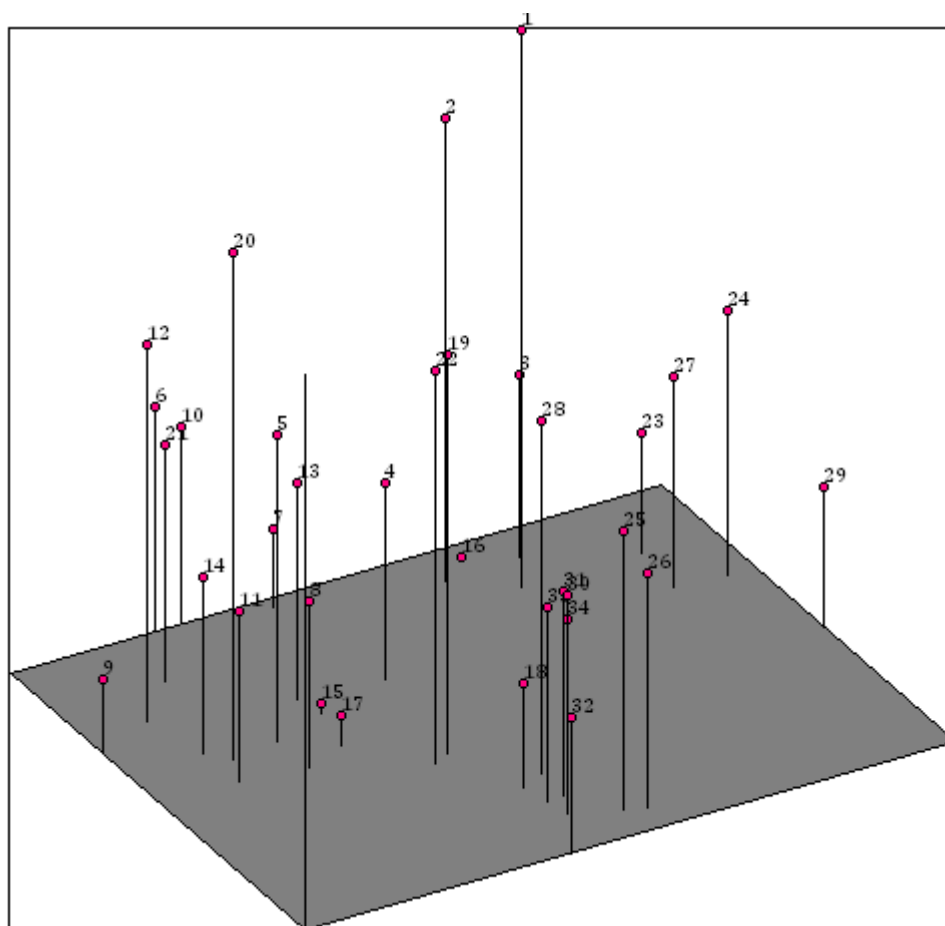
**Fig. 1. Dendrogram of mango germplasm based on RAPD markers**

- |                         |                           |                     |
|-------------------------|---------------------------|---------------------|
| 1 : Banganapalli- 1     | 13. Jalal                 | 25: Paparao Goa     |
| 2 : Banganapalli- 2     | 14: Jehangir              | 26: Peddarasam      |
| 3 : Banganapalli- 3     | 15: Kolanka Goa           | 27: Panukula Mamidi |
| 4 : Banglora- 1         | 16: Kottapalli Kobburi    | 28: Royal special   |
| 5 : Banglora- 2         | 17: Kowsuri Pasand        | 29: Rajamanu        |
| 6 : Baramasi            | 18: Nalla Andrews         | 30: Sora Mamidi     |
| 7 : Cherukurasam        | 19: Nalla Rasalu          | 31: Suvarnarekha    |
| 8 : Chinnarasam         | 20: Navaneetham           | 32: Tella Gulabi    |
| 9 : Chinna Suvarnarekha | 21: Nuzividu Tiyya Mamidi | 33: Tella Rasalu    |
| 10: Elamandala          | 22: Nuzividu Rasalu       | 34: Rajamamidi      |
| 11: Hyder               | 23: Panchadara Kalasa     |                     |
| 12: Imam Pasand         | 24: Pandurivari Mamidi    |                     |

Genetic similarity based on Jaccard's coefficient revealed the considerable level of diversity among the germplasm under study. The average genetic similarity among thirty four mango germplasm ranged from a coefficient of 0.67 to 0.88 with an average similarity coefficient of 0.78 among the group of germplasm studied. All the 34 mango germplasm were grouped into two clusters of which 29 germplasm were grouped into cluster I and the remaining

5 germplasm grouped into cluster II. Germplasm in these two clusters diverged at a genetic similarity value of 0.67 (**Fig. 1**). The description of the data using three dimensional pictorial graphs was presented in **Fig. 2**.

Cluster I was further divided into sub cluster IA with 19 germplasm and sub cluster IB with 10 germplasm at a similarity coefficient value of 0.70 as shown in the



**Fig. 2. Relative position of mango germplasm based on RAPD markers (Three dimensional)**

- |                         |                           |                     |
|-------------------------|---------------------------|---------------------|
| 1 : Banganapalli- 1     | 13. Jalal                 | 25: Paparao Goa     |
| 2 : Banganapalli- 2     | 14: Jehangir              | 26: Peddarasam      |
| 3 : Banganapalli- 3     | 15: Kolanka Goa           | 27: Panukula Mamidi |
| 4 : Banglora- 1         | 16: Kottapalli Kobbari    | 28: Royal special   |
| 5 : Banglora- 2         | 17: Kowsuri Pasand        | 29: Rajamanu        |
| 6 : Baramasi            | 18: Nalla Andrews         | 30: Sora Mamidi     |
| 7 : Cherukurasam        | 19: Nalla Rasalu          | 31: Suvarnarekha    |
| 8 : Chinnnarasam        | 20: Navaneetham           | 32: Tella Gulabi    |
| 9 : Chinna Suvarnarekha | 21: Nuzividu Tiyya Mamidi | 33: Tella Rasalu    |
| 10: Elamandala          | 22: Nuzividu Rasalu       | 34: Rajamamidi      |
| 11: Hyder               | 23: Panchadara Kalasa     |                     |
| 12: Imam Pasand         | 24: Pandurivari Mamidi    |                     |

dendrogram. The sub cluster IA was further sub divided into two groups IAa with 7 germplasm and IAb with 12 germplasm at a similarity index of 0.71 in the dendrogram. The germplasm in IAa was further divided into two groups. IAa1 with 6 germplasm and IAa2 with (Baramasi) at a similarity index of 0.73 in the dendrogram.

The IAa1 group was further divided into 3 clusters, the cluster IAa1a with the germplasm Banganapalli – 1 & 2 were straddling the extreme at similarity index of 0.87 in the dendrogram, in cluster IAa1b, the germplasm Banganapalli -3 and Banglora - 2 at similarity index of 0.78 in the dendrogram while in cluster IAa1c, the germplasm Banglora -1 and Cherukuram have spanned the extremes at similarity index of 0.83 in the dendrogram.

The IA- b group was further divided into two groups. IAb1 with 5 germplasm and IAb2 with 7 germplasm at a similarity index of 0.75 in the dendrogram. The IAb1 group was further divided into 2 clusters, IAb1a and IAb1b as a solitary cluster (Rajamamidi). Cluster IAb1a was further divided into sub cluster IAb1a1 with three germplasm and sub cluster IAb1a2 with one cultivar (Panukula Mamidi). Sub cluster IAb1a1 with three germplasm (Sora Mamidi and Tella Rasalu) as a cluster and Jalal as a solitary cluster at a similarity index of 0.81 in the dendrogram.

The IAb2 group was further divided into 2 clusters, IAb2a and IAb2b. Cluster IAb2a consists of (Nalla Rasalu) as a solitary cluster at similarity index of 0.82 in the dendrogram, Panchadara Kalasa and Pandurivari Mamidi at similarity index of 0.84 in the dendrogram, Navneetham at similarity index of 0.82 in the dendrogram and Paparao Goa and Peddarasam at similarity index of 0.81 in the dendrogram. Cluster IAb2b with Royal Special as a solitary cluster at a similarity index of 0.79 in the dendrogram.

The germplasm in the IB was further divided into two clusters IBa and IBb. IBa with Chinnarasam and Jehangir at similarity index of 0.76. IBb was further divided into two groups, IBb1 and IBb2. Cluster IBb1 was further divided into IBb1a with Elamandala and Imam Pasand as a cluster at a similarity index of 0.81, Chinna Suvarnakha as a solitary cluster at a similarity index of 0.77, and IBb1b with Kolanka Goa at a similarity index of 0.73 in the dendrogram. The second group IBb2 consisting of two clusters IBb2a and IBb2b, cluster IBb2a with Hyder and Kottapalli Kobbari as a cluster at similarity index of 0.84, Nalla Andrews as a solitary cluster at similarity index of 0.78 and cluster IBb2b with Kowsuri Pasand as a solitary cluster at similarity index of 0.76 in the dendrogram.

Cluster II was further divided into sub cluster IIA with two germplasm and sub cluster IIB with three germplasm at a similarity coefficient value of 0.68 as shown in the dendrogram. The sub cluster IIA consists of Nuzividu Tiyya Mamidi and Nuzividu Rasalu at a similarity index of 0.74 in the dendrogram. The germplasm in IIB was further divided into two groups. IIBa with Rajamanu and

Suvarnakha at similarity index of 0.74, and IIBb with Tella Gulabi as a solitary cluster at similarity index of 0.72 in the dendrogram.

The accessions within the cultivar i.e., Banganapalli -1, Banganapalli -2 and Banginapalli-3 showed genetic similarity which is in good agreement from a single ancestor with their putative pedigrees. The germplasm Panukula Mamidi and Rajamamidi were closely clustered since these two germplasms are native to the Vizianagaram district. Similarly, the germplasm Nuzividu Tiyya Mamidi and Nuzividu Rasalu occurred in the same cluster indicated that these germplasms also originated from the same geographical area, it can be concluded that they were separated into the cluster based on geographic origin. Region-based grouping of Indian germplasm was earlier reported by Lopez *et al.* (1997) and Ravishankar *et al.* (2000) in mango.

The results of the present study indicated that RAPD markers can be employed in fingerprinting, characterization of germplasm, assessment of molecular genetic divergence and relatedness among mango germplasm. This information could be used successfully for cultivar identification and for the assessment of genetic diversity among mango germplasm.

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