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Rani S
Assistant Professor, Sant Baba
Bhag Singh University,
Jalandhar, Punjab, India

Sharma JR
Sr. Scientist Department of
Horticulture, CCS Haryana
Agricultural University, Hisar,
Haryana, India

Sehrawat SK
Professor Department of
Horticulture, CCS Haryana
Agricultural University, Hisar,
Haryana, India

Correspondence
Rani S
Assistant Professor, Sant Baba
Bhag Singh University,
Jalandhar, Punjab, India

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Standardization the protocol for high genomic DNA yield and quality for guava cultivars

Rani S, Sharma JR and Sehrawat SK

Abstract

DNA is the basic requirement for genomic analysis in plants and animals so its quality must be high for accurate result. DNA extraction in recalcitrant guava is difficult because of the high polyphenols, tannins and polysaccharides present in the guava leaf tissues. Therefore, a modified protocol is present here, which is improvement over the existing over the CTAB method. It involves double extraction using 3.5 % CTAB in the initial cell lysis, followed by RNase treatment. The highest amount of DNA per gram of fine ground leaf tissue was in L-49 extracted using modified CTAB method (485.2µg/ml of leaves) followed by Allahabad Safeda (398.1µg/ml of leaves) and Chinese guava (380.5 µg/ml of leaves). The superior quality DNA from thirteen guava cultivars and two species evident from the purity Indies. The absorbance ratio of 1.74 to 1.8 (A260/A280), was recorded which reflects the quality of isolated DNA.

Keywords: Guava (*Psidium guajava* L.), leaf tissues, DNA, CTAB method

Introduction

Guava (*Psidium guajava* L.) is a luscious and important tropical fruit crop. It belongs to family Myrtaceae and originated in tropical America perhaps from Mexico to Peru. It was introduced to India by the Portuguese during the early 17th century (Hayes, 1957) [1]. Guava is the hardiest among tropical fruit trees and excels most other fruit crops in productivity and adaptability. It scores over other fruits in ascorbic acid, pectin and other mineral contents. Therefore, it is aptly referred to as 'Poor man's Apple' and 'Apple of the Tropics'. In India, it ranks fourth in total cultivated area after mango, banana, citrus. Uttar Pradesh is the largest producer of guava in India and Allahabad has the reputation of producing the world's best guavas (Mitra and Bose, 1990) [2]. It being the primary centre for origin of the species and in order to protect these biodiversity resources, there is an urgent need for development of fingerprints. To overcome the various drawbacks associated with morphological characterization, it is now widely accepted that information generated from DNA based polymorphisms provides the best estimate of genetic diversity. Molecular techniques have been widely used for assessing the genotypic profiles, genetic diversity and cultivar identification in many plants (Anand, 1999) [3] for any molecular studies, the prerequisite is the superior quality genomic DNA without any contamination of RNA, proteins and metabolites. In perennial fruits trees, leaf tissues are rich in polysaccharides, polyphenols and tannins as reported in apple (Kim *et al.*, 1997) [4], mango (Kit and Chandran, 2004) [5] and litchi (Puchooa *et al.*, 2004) [6]. Thus, in tissues with higher levels of polysaccharides and polyphenols, genomic DNA extraction becomes very difficult. For plant genomic DNA isolation the common methods (Cetyl trimethyl ammonium bromide, CTAB method) and Dellaporta *et al.* (1983) [7] (Potassium acetate or Dellaporta method). Many variants of CTAB method have been developed for different perennial crops, *viz.*, mango (Kit and Chandran, 2010) and litchi (Ding *et al.*, 2000) [8]. Though, these protocols resulted in good quality DNA in guava, over time there was oxidation of phenolic compounds that further inhibited PCR reactions. The disturbances due to excess phenols being co-isolated with DNA has been highlighted earlier by different workers (Jiang, 2000) [9]. This protocol which not only resulted in higher DNA yield but also superior quality DNA over already established protocols.

Materials and Methods

Guava cultivars viz., 1-Hisar Surkha, 2-Hisar Safeda, 3- Strawberry, 4-Chinese guava, 5- Supreme, 6-Lalit, 7-Banarasi Surkha, 8- Lucknow-49, 9- Shweta, 10-Allahabad Safeda, 11-Super Acid, 12- SP 6550, 13- Patillo, 14-Hybrid Red Superme, 15-Super max Ruby, taken from orchard of department of horticulture, cshau Hisar were used for the study. The DNA isolation was done following the CTAB protocol with modifications. Five gram of leaf tissues was powdered using liquid nitrogen along with 2% of PVP, homogenized with 15 ml of 3.5 X CTAB extraction buffer (3.5% CTAB, 100mM Tris-HCl [pH 8.0], 20mM EDTA [pH-8.0], 1.4M NaCl, 2% mercaptoethanol, 2% Polyvinyl pyrrolidone), which was preheated at 65°C for 30 min and incubated at 65°C water bath for 2 hrs with occasional mixing. Equal volume of chloroform: isoamyl alcohol (24:1) was added and incubated for 20 min at room temperature (RT). The upper aqueous layer was extracted after centrifugation at 10,000 rpm for 15 min at RT. Pellet was resuspended in 5 ml of 3.5X CTAB for double extraction and all the above steps were repeated. To the upper aqueous phases pooled together, 2/3rd volume of isopropanol was added and incubated for 1 hr at 4°C. The DNA was pelleted, washed with 70% ethanol, air dried and rehydrated in 600µl of elution buffer (TE buffer). About 5µl RNaseA (1 mg/ml) for every 100µl DNA sample was added and incubated in 37°C for 1 hr to degrade RNA contamination. Then the sample were purified by phenol: chloroform: isoamyl alcohol (25:24:1) purification and re-extracted with 1/10th volume of 3M sodium acetate (pH5.2) and 0.6 volume of ice cold isopropanol.

UV-Spectrophotometric estimation of DNA quantity and quality

For DNA quantity estimation, an aliquot of DNA samples was suitably diluted and absorbance (A) was determined at 260 nm in a spectrophotometer. Using the relationship of O.D. unit of 1.0 at 260 nm equivalent to 50µg DNA per ml, the quantity of DNA was estimated from the following formula: Concentration of DNA (µg/ml) = A₂₆₀ x 50 x dilution factor

Quality of DNA

Quality of DNA sample was checked by UV spectrophotometer by determining absorbance at wavelengths i.e. 260 nm and 280 nm the ratio of the absorbance at 260 nm and 280 nm was noted. A₂₆₀ / A₂₈₀ = 1.8 (pure DNA)

Quality of DNA precipitation was also tested by submerged horizontal agarose (0.8%) gel electrophoresis.

Results and Discussion

DNA extraction

Pure and RNA free high molecular weight DNA was isolated in the range of 229.1 µg/ml to 485.2 µg/ml.

Modified CTAB method

The highest amount of DNA per gram of fine ground leaf tissue was in L-49 extracted using modified CTAB method (485.2µg/ml) followed by Allahabad Safeda (398.1µg/ml) and Chinese guava (380.5 µg/ml) (Table 1). The absorbance ratio of 1.8(A₂₆₀/A₂₈₀), was recorded which reflects the quality of

isolated DNA.

Electrophoretic pattern of purified genomic DNA of guava genotypes with modified CTAB method is shown in Plate 1. Evolutionary relationships, biochemical networks, Genetic diversity, genome sequencing, numerical taxonomy and molecular systematics are dependent on good quality DNA as a base or starting material (Kumar *et al.* 2012) [10]. For such molecular studies, the first and foremost requirement is a superior quality of DNA which depends on DNA extraction procedures. Classical CTAB method of DNA extraction yielded good quality DNA in guava, but the yield was low and slowly oxidation of phenolic compounds occurred which inhibited PCR reactions. The isolation of genomic DNA from plants containing polysaccharides, phenolics, terpenoids, tannins and metabolites is cumbersome because these compounds binds with DNA and get co-extracted with DNA (Micheils *et al.*, 2003 and Puchooa, 2004) [11]. Polysaccharides interfere with biological enzymes such as polymerases, restriction endonucleases and ligases (Micheils *et al.*, 2003). A wide range of antioxidants are used during DNA extractions to reduce the phenolic contaminations which include: β-mercaptoethanol, polyvinyl pyrrolidone (PVP) and poly vinyl polypyrrolidone (PVPP) (Kim *et al.*, 1997; and Puchooa, 2004). Among these chemicals, PVP (MW, 10000 or 40000) at 1% to 6% concentration is most commonly used for reducing phenolic contamination in the extractions in litchi (Puchooa, 2004, Li and Zhang, 2004) [12]. Use of PVP (2.0% V/V) and 3.5% CTAB (instead of 2% as in classical CTAB method), phenolic and polysaccharide contaminations in DNA were reduced in the guava samples in the present study. PVP forms complex hydrogen bond with latex lactone, lactucin and other phenolics and coprecipitates with cell debris upon lysis. When the extract is centrifuged in presence of chloroform, the PVP complex accumulated at the interface between the organic and the aqueous phase (Aljanabi *et al.*, 1999, Kim *et al.* 1997 and Michiels *et al.*, 2003) [13]. CTAB binds the fructans and other polysaccharides and forms complex that are removed Washing with ethanol was critical for washing impurities as well as recovery of DNA as a lot of degraded nucleic acids and small species that influence A₂₆₀ values as this is critical determinant of DNA concentration and yield. On 0.8% agarose gel, a brighter intact band was visualized in all thirteen guava cultivars and two species without any RNA or protein contamination. This was further confirmed by UV-spectrophotometric analysis in which the DNA concentration and quality based on purity index (A₂₆₀/A₂₈₀) values matched with the visual quantitation and quality analysis on agarose gel.

DNA concentration, yield and quality

In guava, using the present protocol, DNA concentration was found to be varying in range of 229.1-485.3µg/ml with mean being 317.9µg/ml. The highest concentration of DNA was observed in Lucknow-49 (485.3µg/ml) while the lowest concentration was found in Hisar Surkha 229.1 µg/ml.

The DNA concentration recorded was comparable with earlier reports in plants having high phenol content like, litchi (Kumar *et al.* 2012) sweet potato, oil palm and other dicotyledonous plants (Ganeshiah *et al.*, 2000) [14].

Table 1: Quantity and quality of genomic DNA isolated from guava genotypes using modified CTAB method

S. No.	Genotype	Quantity of DNA (µg/ml)	Ratio of A ₂₆₀ /A ₂₈₀
1	Hisar Surkha	229.1	1.8
2	Hisar Safeda	298.1	1.78
3	Strawberry	234.2	1.77

4	Chinese guava	380.5	1.8
5	Supreme	276.2	1.74
6	Lalit	380.1	1.8
7	Banarasi Surkha	320.8	1.76
8	Lucknow-49	485.2	1.79
9	Shweta	312.7	1.8
10	Allahabad Safeda	398.1	1.8
11	Super Acid	267.3	1.77
12	SP6550	299.5	1.76
13	Patillo	278.2	1.79
14	Hybrid Red Supreme	299.1	1.75
15	Super Max Ruby	310.2	1.8

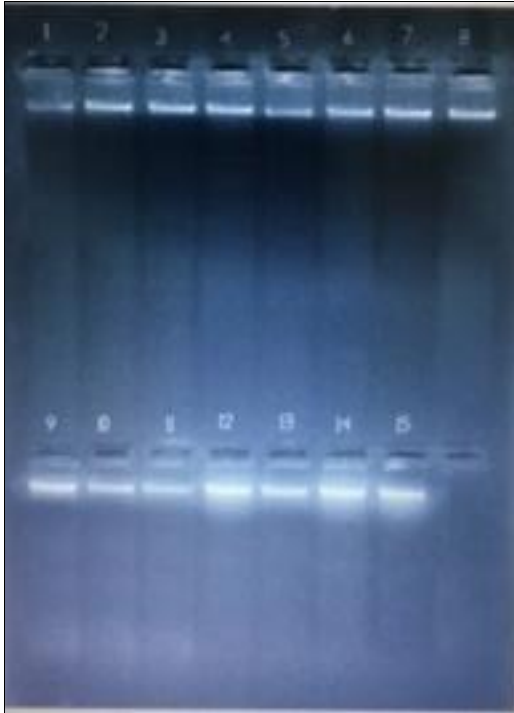


Plate 1

Agarose Gel Profile of genomic DNA of thirteen guava cultivars and two species viz., 1-Hisar Surkha, 2-Hisar Safeda, 3-Strawberry, 4-Chinese guava, 5- Supreme, 6-Lalit, 7-Banarasi Surkha, 8- Lucknow-49, 9- Shweta, 10-Allahabad Safeda, 11- Super Acid, 12- SP 6550, 13- Patillo, 14-Hybrid Red Superme, 15-Super max Ruby. Higher yield and Intact DNA is observed on 0.8 % agarose gel

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