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Short communication

Comparison of mango genomic DNA isolation methods for next generation sequencing

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ABSTRACT

Six different methods of DNA isolation namely, CTAB, CTAB with PVP, Qiagene DNA extraction Mini and Maxi kits, CTAB with addition of PVP and purification using PCI followed by Qiagen Genomic-tip 500/G were compared for recovery of quality DNA from mango leaves. The higher yield (1375.0 ng/ µl) of good quality ($A_{260/280}$ and $A_{260/230}$ 1.80 & 1.90, respectively) DNA was obtained with modified CTAB method with addition of PVP (MW, 40,000) followed by purification using phenol: chloroform: isoamylalcohol 25:24:1 and Qiagen Genomic-tip 500/G as compared to standard CTAB method (1096.50 ng/ µl; $A_{260/280}$ and $A_{260/230}$ 1.40 and & 1.10, respectively). The DNA obtained using modified CTAB method was found suitable for PCR, PacBio, ddRAD sequencing and long-term storage.

Key words: DNA extraction, ddRAD sequencing, *Mangifera indica* L.

Mango is a major fruit crop of the tropics and subtropics, particularly in India, and considered as the 'King of fruits' (Mukherjee *et al*., 4). India is the largest producer of mango in the world with 15.03 mt production from an area of 2.31 m ha. The genome resource wealth of mango is extremely meager and limited genomic information is available for understanding the genetics of useful horticultural traits. Therefore, it is imperative to improve the genome resources of mango by way of whole or segmental genome sequencing. Isolating high quality DNA is essential for generation of shotgun and pair-end next generation sequence data on mango genome using Roche 454 and Illumina (MiSeq and HiSeq), additional SOLiD, PacBio sequencing technologies, which will be further used for improving the mango genome assembly (Barabaschi *et al*., 1). Different methods are available for the isolation of genomic DNA from plant tissues. In general, all methods involve disruption and lysis of the starting material followed by the removal of polysaccharides, proteins and other contaminants, and finally recovery of the DNA. Several protocols for removing polysaccharides from plant tissues during DNA isolation have been reported (Puchooa, 5; Sharma *et al*., 6). Mango is considered difficult fruit crop for DNA isolation due to hardiness, higher fibrous material and large amount of phenolic compounds (Uddin *et al*., 7). Hence, the standardization of protocol for DNA isolation of mango is the most critical step. In the present investigation different DNA isolation methods have been compared for higher yield of quality DNA from mango leaves.

Leaf samples from 48 mango varieties were collected from the Main Orchard of mango at the Division of Fruits and Horticultural Technology, IARI, New Delhi in the month of March, 2013. Initially, genomic DNA from mango leaves was extracted from 15 g of each of the two samples by CTAB method as described by Doyle and Doyle (3). In order to enhance the yield of quality DNA, modifications like addition of 1% of PVP (Polyvinylpyrrolidone, MW 40,000) was done. The ground leaf samples were transferred to 15 ml extraction buffer containing 2% w/v CTAB, 1.4 M NaCl, 20 mM EDTA, 0.1% β-mercaptoethanol, 100 mM Tris (pH 8.0) pre-heated to 65°C and incubated at 65°C for 1 h with occasional shaking. The homogenate was cooled to room temperature and extracted with 15 ml of chloroform: isoamyl alcohol (24:1), centrifuged at 12,000 rpm for 15 min. The clear aqueous phase was separated and 5 ml of 5M NaCl and 10 ml isopropanol were added and stored at 4°C overnight. This was again centrifuged at 12,000 rpm for 15 min. and the supernatant was decanted retaining the pellet. The pellet was air dried. Then pellet was dissolved in 500 µl of TE and left for 10 min. For removing RNA 5 µl RNase was added per ml of DNA and incubated for 1 h at 37°C. For ddRAD sequencing DNA was extracted using DNeasy Plant Mini Kit, Qiagen and also for higher yield DNeasy Plant Maxi Kit. For generating PacBio library, DNA was isolated with CTAB method with addition of 1% PVP. To obtain ultra pure DNA, it was purified with phenol: chloroform: isoamyl alcohol (25:24:1)

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and Qiagen Genomic-tip 500/G. The integrity of DNA was assessed by agarose gel analysis. The quantitation of DNA was done by using a UV-VIS spectrophotometer (UNICAM) and absorbance (A) was recorded at 260, 230 and 280 nm. The quantity and quality of DNA yielded in six different methods are given in Table 1.

Standard CTAB method followed for genomic DNA extraction resulted in good yield (1096.50 ng/ µl**)** but of inferior quality DNA ($A_{260/280}$ and $A_{260/230,}$ 1.40 and 1.10, respectively). To improve the quality and extraction efficiency, addition of PVP along with standard protocol was attempted. It enhanced the quantity (1275.9 ng/ µl) of the DNA that is for average of two samples. The genomic DNA extracted from DNeasy Plant Mini Kit, Qiagen yielded low quality ($A_{260/280}$ and $A_{260/230}$ 1.60 & 1.10, respectively) and less quantity (42.2 ng/ µl) DNA. Further, DNeasy Plant Mini Kit was used for extraction of DNA from 48 mango varieties for ddRAD sequencing. Average DNA yield among mango varieties varied from 25-40 ng/ µl and quality ratio ($A_{260/280}$) varied from 1.6- 1.7 (Table 2). Further, the yield of DNA was enhanced (200.4 ng/ µl) with DNeasy Plant Maxi Kit but quality of DNA was poor ($A_{260/280}$ and $A_{260/230}$, 1.55 and 1.10, respectively). The maximum DNA yield (1450.0 ng/ µl) was obtained with standard CTAB method with addition of PVP followed by purification using phenol: chloroform: isoamyl alchol in the ratio of 25:24:1 (v/v/v) but quality of DNA was average ($A_{260/280}$ and $A_{260/230}$, 1.51 & 1.60, respectively). Further, the quality of DNA was improved to acceptable level ($A_{260/280}$ and $A_{260/230}$, 1.80 & 1.90) by use of Qiagen Genomic-tip 500/G. The purified high quality DNA was used further for PacBio sequencing. The genomic DNA extracted by different methods like CTAB, CTAB with addition of PVP, DNeasy plant Mini and Maxi kits (Qiagen), CTAB with addition of PVP followed by purification using both PCI and Qiagen genomic tip 500G had their own advantages and disadvantages. CTAB with addition of PVP followed by purification using PCI and Qiagen genomic tip 500G method resulted higher yield of purified DNA as compared with other methods.

During the last 20 years, numbers of protocols for DNA extraction from plant samples have been reported like DNA precipitated in salt solution with sodium acetate for phenol-chloroform methods, isopropanol for Kit Wizard™ Genomic DNA Purification (Promega), SDS method, CTAB method,

Table 1. DNA quantitation and quality analysis in mango.

Method	A_{260}/A_{280}	A_{260}/A_{230}	DNA yield (ng/µl)
CTAB	1.40	1.10	1096.50
CTAB and PVP	1.41	1.11	1275.90
DNeasy Plant Mini Kit, Qiagen	1.60	1.10	42.20
DNeasy Plant Maxi Kit, Qiagen	1.55	1.10	200.40
CTAB + PVP + PCI	1.51	1.60	1450.00
CTAB + PVP + PCI + Qiagen Genomic-tip 500/G	1.80	1.90	1375.00

Table 2. DNA quantitation and quality analysis of 48 mango varieties using Qiagen DNeasy plant mini kit for ddRAD sequencing.

S. No.	Variety	A_{260}/A_{280}	DNA (ng/µl)	Total DNA yield (µg)
1	St. Alexandrina	1.7	40	12.0
2	Edward	1.7	40	12.0
3	Pusa Surya	1.6	41	12.3
4	Ameitista	1.6	27	8.0
5	Tommy Atkins	1.6	40	12.0
6	Willard	1.6	35	10.5
7	Ratna	1.6	35	10.5
8	Sensation	1.6	40	12.0

Contd...

Table contd...

S. No.	Variety	A_{260}/A_{280}	DNA (ng/µl)	Total DNA yield (µg)
9	Bhadauran	1.7	35	10.5
10	Primor de Amoreira	1.7	35	10.5
11	Mahmood Vikarabad	1.7	27	8.0
12	Janardan Pasand	1.7	27	8.0
13	Zardalu	1.7	35	10.5
14	Bombay Green	1.6	40	12.0
15	Kala	1.7	27	8.0
16	Alphan	1.7	27	8.0
17	Husnara	1.7	40	12.0
18	Mombasa	1.7	27	8.0
19	Machhli	1.7	27	8.0
20	Hardil Aziz	1.7	27	8.0
21	Gulab Khas Green	1.7	35	10.5
22	Khasulkhas	1.7	27	8.0
23	Sonatol	1.7	40	12.0
24	Samar Bahist Alibagh	1.7	35	10.5
25	Alphanso	1.7	30	9.0
26	Amrapali	1.7	35	10.5
27	Chausa	1.6	25	7.5
28	Dushehari	1.7	30	9.0
29	Langra	1.7	25	7.5
30	Mallika	1.7	25	7.5
31	Neelum	1.7	25	7.5
32	Pusa Arunima	1.7	25	7.5
33	Pusa Lalima	1.7	25	7.5
34	Pusa Pratibha	1.7	30	9.0
35	Pusa Shresth	1.7	30	9.0
36	Rataul	1.6	30	9.0
37	Extrema	1.7	30	9.0
38	Hyb. 165	1.7	25	7.5
39	Irwin	1.7	30	9.0
40	Iturba	1.7	30	9.0
41	Kurukkan	1.7	30	9.0
42	Olour	1.7	30	9.0
43	Safdar Pasand	1.7	30	9.0
44	Ramkela	1.7	30	9.0
45	Rosari	1.7	35	10.5
46	Pusa Peetamber	1.7	35	10.5
47	Smith	1.7	40	12.0
48	Zill	1.7	30	9.0

and ethanol for Sarkosyl method (Puchooa, 5). Plant that had high polyphenolic content can use phenol that work together with SDS to extract it. DNA extraction for PacBio sequencing has two requirements, (i) extraction of high molecular weight DNA, and (ii) extraction of DNA free from inhibitors for subsequent molecular studies (Bertrand *et al*., 2). Genomic DNA isolation protocol of mango was optimized with modifications made in standard CTAB method of Doyle and Doyle (3) and good quality and considerably higher yield was obtained in the method involving CTAB and 1% PVP followed by purification using PCI and Qiagen genomic tip 500G in the present study. From this study it was concluded that for isolating high quality DNA for generation of shotgun and pair-end next generation sequence data on mango genome, CTAB method is good for genomic DNA isolation but further DNA quality and quantity improvement with addition of PVP followed by purification of isolated DNA is important.

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