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Suhas B Jadhav
Assistant Professor, College of Agriculture, Ambajogai, Beed, Maharashtra, India

Shruti Sunil Jadhav
B. Tech Scholar, School of Biotechnology and Bioinformatics, D.Y. Patil University, Navi Mumbai, Maharashtra, India

Corresponding Author:
Shruti Sunil Jadhav
B. Tech Scholar, School of Biotechnology and Bioinformatics, D.Y. Patil University, Navi Mumbai, Maharashtra, India

Standardization protocol of DNA isolation from *Beta vulgaris*

Suhas B Jadhav and Shruti Sunil Jadhav

Abstract
Beet is an important vegetable which is commonly used in the world. Beet is also known as red beet or garden beet. A simple and efficient protocol for isolating genomic DNA from fresh rhizome of *Beta vulgari* was developed. Three extraction buffers were tested for CTAB method of DNA isolation from rhizome slice of *Beta vulgaris*. Modified CTAB procedure using extraction buffer 3% CTAB, 1% β-mercaptoethanol, 1.4 M NaCl and 2% PVP. The quantity and purity of isolated DNA was higher when extraction buffer 3 was used in CTAB method compared with other two extraction buffer. Amplified product were obtained when isolated DNA was screened with four RAPD primer.

Keywords: DNA isolation, Extraction buffer, CTAB, Genomic DNA

Introduction
Beet is an important vegetable which is commonly used in the world. Beet is also known as red beet or garden beet. Common name of beet is beet, beet (with sugar beet, swiss chard, and spinach beet). Beet should be stored at 40 ^{0}c with a relative humidity of 90-100%. In an acid environment the colour pigment are more stable than at a high pH. Today, beetroot is grown in many countries worldwide, is regularly consumed as part of the normal diet, and commonly used in manufacturing as a food colouring agent known as E162 (Georgiev V.G. *et al.* 2010) [3].
With the increasing use of molecular breeding in plant and its potential use in herbal drug industry, the preparation of good quality and quantity DNA has become a major concern. The extraction of DNA from tissue needs to be simple, rapid, efficient and inexpensive when many samples DNA extraction needed for the population studies, molecular breeding and screening of raw herbal drug materials. Number of methods was developed for extracting plant DNA from different plant parts including roots (Dellaporta *et al.*, 1983; Keim *et al.*, 1988; Doyle and Doyle *et al.*, 1990; Khanuja *et al.*, 1999; Kumar *et al.*, 2003) [1, 5, 2, 6, 7]. The methods employed for extracting DNA from fresh and dried root samples of medicinal plants however are time consuming and yield DNA in lesser quantity and low purity due to the presence of high levels of polysaccharides, phenolics and other secondary metabolites in these samples. The development of molecular technique for genetic analysis had led to a great augmentation in our knowledge of crop genetics and our understanding of the structure and behavior of various crop genomes. These molecular techniques in particular the application of molecular markers, have been used to scrutinize DNA sequence variation in and among the crop species and create a new source of genetic variation by introducing new and favorable traits from related crop species.
In this communication, we have described an easy and rapid protocol to extract genomic DNA from the fresh and dry roots of some important medicinal plants. The method involves a modified CTAB procedure of Doyle and Doyle (1990) [2]. The DNA obtained through this method was highly pure and proved to be good for polymerase chain reaction conducted with random primers. The time taken for DNA extraction using this protocol was also less.

Materials and Methods
The Present investigation was carried out in collaboration with School of Biotechnology and Bioinformatics, D. Y. Patil University, New Mumbai, State-Maharashtra

Plant material: Rhizomes of beet were collected from local vegetable market of Mumbai for DNA isolation fresh slice of rhizome sample of beet were used.

Isolation of DNA

DNA was isolated from fresh thin slice of beet using Cetyl Trimethyl Ammonium Bromide (CTAB) protocol. For isolation of DNA three different type of extraction buffer were prepared for obtaining pure quality DNA.

Extraction buffer

Table 1: Three different types of CTAB Extraction buffer

Buffer solution	Composition	
Extraction buffer-I	PVP	1%
	CTAB	1.5%
	Tris base	130 Mm
	EDTA	16 Mm
	β-mercaptoethanol	0.2%
	Nacl	1.0 M
Extraction buffer-2	PVP	1.5%
	CTAB	2%
	Tris base	120mM
	EDTA	18m
	β-mercaptoethanol	0.5%
	Nacl	1.2M
Extraction buffer-3	PVP	2%
	CTAB	3%
	Tris base	100mM
	EDTA	20mM
	β-mercaptoethanol	1%
	Nacl	1.4M

Protocol for DNA isolation by CTAB method

- Fresh slice of rhizome sample of beet was crushed in mortal and pestle to obtain the fine slurry.
- Add 400µl pre warmed (60°C) extraction buffer into it and was mixed by inversion.
- Mixture was incubated for 60 min at 65°C in hot water bath or thermo mixer.
- Add equal volume of Chloroform: Isoamyl alcohol (24:1) was added each tube and mixed gently but thoroughly to proper mixing of both components, (extraction buffer-400µl=C:I-400µl).
- Centrifugation was carried out 10,000rpm for 10 min at 4°C
- The upper supernatant was transferred into a new 1ml eppendorf tube with a micropipette.
- 0.6 volume of ice-cold isopropanol was added.
- Tubes were kept in refrigerator for 4°C about at 1/2 hrs.
- After that, the sample was centrifuged at 10,000rpm at 4°C for 10 min.
- After centrifugation a pellet was formed at the bottom of the eppendorf tubes.
- The supernatant was removed and the pellet was washed with 70% ethanol.
- The pellet was air dried for 30 min and then dissolved in 100µl of TE buffer.
- The pellet was allowed to dissolve completely overnight at 4°C without agitation.

Quantification of DNA

1. 10ul of DNA was diluted with 90µl water to make 1: 10 dilution. These diluted DNA was read in spectrophotometer to note its reading at 230nm, 260nm, 280nm.
2. Quantity of DNA was calculated by multiplying the value obtained at 260nm with 50 as 1.0 OD at 260nm implies 50ng /µl DNA.
3. Ratio of OD's at 260/280 gives an indication of protein contamination in the preparation ideal value of these ratio should be 1.8.
4. Ratio of OD's at 260/280 gives an indication of other contamination in the preparation.

DNA Confirmation

The DNA obtained after extraction was conformation by running it on 0.8% agarose gel in horizontal gel electrophoresis system.

3µl of genomic DNA template of each genotype loading dye was loaded in the each well.

After completion of running the gel was observed under U V light with help of biorad gel documentation system and DNA quality was confirmed.

PCR amplification

PCR amplification carried out by using RAPD primer for testing quality of DNA for PCR reaction.

Table 2: The list of RAPD (primer) markers used for amplification of the genomic DNA

Sr. No.	Primer Code	Sequence	Annealing Temp. (°C)
1.	OPO05	5'CCCAGTCACT3'	32°C
2.	OPO07	5'CAGCACTGAC3'	32°C
3.	OPO13	5GTCAGAGTCC3'	32°C
4.	OPO18	5'CTCGCTATCC3'	32°C

Preparation of reaction mixture for PCR

The PCR was carried but in small reaction tubes containing a reaction volume typically of 25 µ that was inserted into a PCR machine. For PCR reaction, master mix was prepared first. Table 3

Table 3: Master mix for 1X of 25 µl reaction

Master mix	1X
10X Taq polymerase	2.5µl
Mgcl$_2$ (25mM)	1.3µl
dNTPs(10mM)	o.27µl
Taq polymerase (15U/µl)	0.10µl
Sterile distilled water	15.65µl
PCR Reaction	
Master mix volume	20µl
RAPD primer	1 µl
Template DNA (30ng)	2µl
Total Reaction volume	23µl

Procedure for PCR reaction

1. Sterile micro centrifugation tubes were numbered and placed on PCR tube stand.
2. 2µl of DNA was added to each PCR tube fallowed by 19.99µl master mix.
3. 1.5µl of each forward and reverse primers was added in each PCR tube.
4. The samples were mixed by brief centrifugation to bring down the content to tube.
5. PCR was run on the programmable thermal cycler with the following programme.

Table 4: PCR Thermal Cycler Programme

Initial denaturation	95°C	5 min
45 cycle	94°C denaturation	1 sec
	50°C annealing	2 min
	72°C extension	1 min
Final Extension	72°C	5 min

*Annealing temperature varied from primer to primer.

After completion of the cycles keep the samples at 4°c till electrophoresis.

Result and Discussion

Beet is an important vegetable which is commonly used in the world. Beet is also known as red beet or garden beet. In present investigation CTAB method was used to standardized DNA isolation method from beet. Three different type of extraction buffer were prepared for CTAB method of DNA isolation. The isolation of high-quality DNA is important for all molecular biological analyses, because contaminants such as proteins, polyphenols and polysaccharides can interfere with key enzymes. Thus, it is important (i) to choose the most appropriate part of the plant to use as the source of DNA; and (ii) to establish an optimum extraction protocol to yield high-quality DNA.

Fig 1: DNA isolation from rhizome of beet by using CTAB extraction buffer 3

In extraction buffer 1 C-TAB taken about 1.5%, 0.5% β-mercaptoethanol, 1M NaCl and 1% PVP was used. Whatever DNA obtained was contaminated with RNA and protein and obtained pure quality DNA. In extraction buffer 2, C-TAB concentration used about 2%, 1.5% β-mercaptoethanol, 1.2M NaCl, 1.5% PVP was used in CTAB method of DNA isolation. DNA was obtained less contaminated with RNA and protein compare to extraction buffer 1. In extraction buffer 3 C-TAB concentration 2%, β-mercaptoethanol 1% and NaCl concentration again increased upto 1.4M and PVP was used upto 1%. DNA obtained was good and pure quality and less contaminated with RNA and protein compare to extraction buffer 2. Firstly DNA were isolated from four genotype of beet and load into 0.8% agarose gel. Gel was observed under gel doc under UV rays.

PCR reaction

Isolated DNA of beet was screened by using RAPD primer. Amplified product were obtained when isolated DNA was screened with four RAPD primer *viz.* OPO05, OPO07, OPO13 and OPO18 showed in fig.2

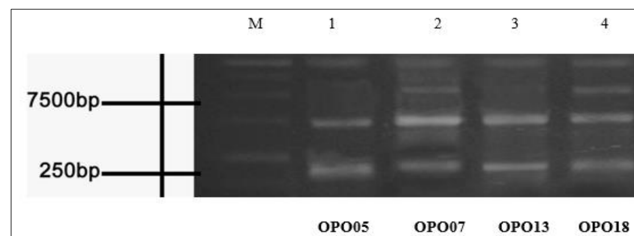

Fig 2: Amplified product of PCR reaction

Discussion

The isolation of high-quality DNA is important for all molecular biological analyses, because contaminants such as proteins, polyphenols and polysaccharides can interfere with key enzymes. Thus, it is important (i) to choose the most appropriate part of the plant to use as the source of DNA; and (ii) to establish an optimum extraction protocol to yield high-quality DNA Kadam *et al*. (2018) [4].

PCR-based multiple loci techniques, which include random amplified polymorphic DNA (RAPD), ISSR and SSR markers, play important roles in crop improvement.

Conclusion

A simple and efficient protocol for isolating genomic DNA from fresh rhizome of *Beta vulgaris* was developed. Three extraction buffer were tested for CTAB method of DNA isolation from rhizome slice of *Beta vulgaris*. Modified CTAB procedure using extraction buffer (3) 3% CTAB, 1% β-mercaptoethanol, 1.4 M NaCl and 2% PVP. Amplified product were obtained when isolated DNA was screened with four RAPD primer *viz*. OPO05, OPO07, OPO13 and OPO18.

References

1. Dellaporta SL, Wood J, Hicks JB. A plant DNA minipreparation: version II. Plant. Mol Biol. Rep 1983;1:19-21.
2. Doyle JJ, Doyle JL. Isolation of plant DNA from fresh tissue. Focus 1990;12:13-15.
3. Georgiev VG, Weber J, Kneschke EM, Denev PN, Bley T, Pavlov AI. Antioxidant activity and phenolic content of betalain extracts from intact plants and hairy root cultures of the red beetroot *Beta vulgaris* cv. Detroit dark red. Plant Foods Hum. Nutr 2010;65:105-111.
4. Kadam SS, Gokhale NB, Sawardekar SV, Burondkar MM, Rasam VG, Rasam DV. Standardization of DNA isolation protocol for banana using rapid method and fidelity testing in tissue culture developed banana plantlet of *Safed velchi*, Environment and Ecology 2018;36(1A):192-196.
5. Keim P, Olson TC, Shoemaker RC. A rapid protocol for isolating soybean DNA. Soybean Genet Newsl 1988;15:150-152.
6. Khanuja SPS, Shasany AK, Darokar MP, Kumar S. Rapid isolation of DNA from dry and fresh samples of plants producing large amounts of secondary metabolites and essential oils. Plant Mol. Biol. Rep 1999;17:74-80.
7. Kumar A, Pushpangadan P, Mehrotra S. Extraction of high molecular weight DNA from dry root tissue of Berberis lycium suitable for RAPD. Plant. Mol. Biol. Rep 2003;21:309a-309d.
8. Ray T, Dutta I, Saha P, Sampa D and Roy SC. Genetic stability of three economically important micro propagated banana cultivars of lower Indo-Gangetic plains, as assessed by RAPD and ISSR markers. Pl Cell, Tissue and Organ Culture 2006;85:11-21.