Nig. J. Biotech. Vol. 33 (2017) 131-135
ISSN: 0189 1731
Available online at
http://www.ajol.info/index.php/njb/index
and www.biotechsocietynigeria.org
DOI: https://dx.doi.org/10.4314/v33i1.19

NIGERIAN JOURNAL OF BIOTECHNOLOGY

The levels of yield and purity of genomic DNA from five tomato cultivars subjected to two DNA extraction techniques

Ganiyu, S. A.[1], Yusuf, S. M.1, Agbolade, J. O.[2] and Imonmion, J. E.[3]

[1]Department of Agronomy, Federal University, Kashere, PMB 0182, Gombe State, Nigeria.
[2]Department of Plant Science and Biotechnology, Federal University, Oye-Ekiti, Ekiti State, Nigeria.
[3]Department of Crop Protection, Federal University of Agriculture, Abeokuta, PMB 2240, Nigeria.

(Received: 09:06:2017; Accepted:29 :06:2017

Abstract

Isolation of good quality genomic DNA from different plant materials is an important prerequisite for many molecular techniques related to both basic and applied research in the areas of plant molecular biology, crop improvement, biodiversity studies and conservation of genetic materials. Therefore, the need to extract reasonably pure DNA of both good quality and quantity for the downstream successful DNA-based diagnostic techniques is required. In this study, yield and purity of the extracted DNAs, using Dellaporta et al. (1983) and the use of Zymo Research Plant/Seed DNA MiniPrep Kit (ZR kit), were quantified with Nanodrop spectrophotometer and on agarose gel after polymerase chain reaction (PCR) amplification. The results of the DNA yield and purity were in the range of 24.42 to 1430.52 ng/µl and 1.11 to 2.00, respectively. The outstanding DNA yield in Kerewa cultivar in yield (1430.52 ng/µl) and purity (1.99) makes it appropriate for downstream DNA-based diagnostic techniques in biotechnology research. On agarose gel DNA bands appeared to be compact with negligible smearing with Dellaporta et al. method indicating good quality of DNA. The results demonstrated that good yield of very pure, intact, high quality genomic DNA could be obtained from tomato leaves using the method of Dellaporta et al.

Keywords: DNA, extraction, purity, tomato, yield.

Correspondence: ganiyu.sikiru@yahoo.com; +234-8136392050.

Introduction

DNA extraction is a routine step in many biological studies including molecular identification, phylogenetic inference, genetics, and genomics. Isolation of good quality genomic DNA from different plant materials is an important prerequisite for many molecular techniques related to both basic and applied research in the areas of plant molecular biology, crop improvement, biodiversity studies and conservation of genetic materials (Kumari et al., 2012). Also, Kumari et al. (2012) identified that apart from quality, overall yield is also important for various applications. Extracted DNA that is pure give good polymerase chain reaction (PCR) products compared to DNA with lower purity which requires several dilutions before it will give a good PCR product (Turaki et al., 2017). Ecology of southwestern Nigeria is very favorable for tomato production. It is an economic fruit vegetable for tomato growers, consumed fresh and also used to manufacture a wide range of processed products. Unfortunately, many cultivars grown are susceptible to bacterial and fungal diseases. One of the most challenging is bacterial wilt caused by *Ralstonia solanacearum*. This disease can constitute up to 100% yield loss in endemic areas (Popoola et al., 2015). Moreover, due to the effect of this destructive disease, most tomato fruits are transported from northern part of the country to southern states and have continued to increase each year. However, breeding for resistance against diseases is very challenging. Breeding a resistant variety using an updated gamplasm as a donor typically requires a series of backcrosses to the cultivated recurrent parent to combing desirable characteristics. This procedure is time consuming. To start with, the application of molecular breeding through extraction

high quality DNA makes it simplified even for numerous samples.

Genetic analyses of plant rely on high yields of pure DNA samples. In Some plant species, DNA extraction is difficult because of contents of polyphenols and other secondary metabolites (Zhang and Stewart, 2000; Dehestani and Tabar, 2007; Sahasrabudhe and Deodhar, 2010; Srivastava et al., 2010; Okpodu and Abdullah-Israel, 2011). During the DNA isolation protocols, higher quality and yield are two important factors. The DNA isolation protocols include some similar steps. The main purpose is to separate DNA from other compounds especially proteins, carbohydrates, phenols and other polysaccharides (Atak et al., 2014). After DNA extraction, it is important to determine purity and yield. The DNA isolated in pure form is used in different molecular studies and different purposes (Varma et al., 2007; Tiwari et al., 2012).

DNA isolation and purification are two important steps for molecular biology studies (Arif et al., 2010). Like reagents, good quality DNA is an essential to achieve good results in experiments, especially in the Polymerase Chain Reaction (PCR), in which excess of cell debris and proteins may inhibit the amplification (Baise et al., 2002). Many molecular biology techniques and investigations require genomic DNA as a starting material (Vaseemuddin, 2010). The degree of purity and quality can vary from plant and isolation protocols. Many different methods and technologies are available for the isolation of genomic DNA (Atak et al., 2014). Different methods have been developed for genomic DNA isolation depending on the nature and complexity of the plant materials (Sharma et al., 2008). Similarly particular leaf textures and types can impede extraction of DNA (Mirbahar et al., 2014). This connotes that different genotypes/cultivars respond differently to methods of DNA isolation. In this study, yield and purity of DNA from five tomato cultivars (F1-Mongal, Pure-water, Santana, Kerewa and Tyre-type) were compared using Dellaporta et al. (1983) and a commercially available Zymo Research Plant/Seed DNA MiniPrep Kit.

Materials and Methods

Experimental site

The experiment was carried out at the International Institute of Tropical Agriculture (IITA), Ibadan. Nigeria.

Plant materials for DNA isolation

The seeds of five tomato cultivars (F1-Mongal, Pure-water, Santana, Kerewa and Tyre-type) were grown under screenhouse conditions and apical leaves were harvested from two-week old seedlings for DNA isolation. Fresh leaf tissue (150 mg) was weighed on an electronic metlar balance and ground into a fine powder in liquid nitrogen with a pestle and mortar, transferred into a 1.5 ml tube and temporarily stored at -20°C.

Genomic DNA Extraction

Dellaporta et al. (1983) and Zymo Research Plant/Seed DNA MiniPrep Kit (ZR kit) were used.

Method 1: DNA isolation procedures followed Dellaporta et al. (1983) with little modification made on the amount of reagents used. To each tube containing ground tissue (150 mg), 700 µl of hot (65° C) plant extraction buffer (PEB)(637.50 ml dd H_2O, 100 ml 1 M Tris-HCl, PH 8.0, 100 ml 0.5 M ethylenediaminetetra acetic acid (EDTA) , PH 8.0, 100 ml 5 M NaCl and 62.50 ml 20% Sodium Dodecyl Sulphate (SDS)) was added. One percent ß-mercaptoethanol was added to the pre-warm PEB just before use. The tubes were capped and inverted gently 6-7 times to mix the sample with the buffer. The solution was incubated at 65°C in water bath for 20 mins with occasional mixing to homogenize the samples. After, it was removed and uncapped. The tubes were allowed to cool at room temperature for 2 mins. 500 µl of 5 M ice-cold potassium acetate (CH_2COOK) was added and recapped, mixed and incubated on ice-cold for 20 mins. It was then spinned at 12,000 rpm for 10 min and supernatant was transferred into new tubes. Chloroform isoamyl (700 µl) was added and spinned at 10,000 rpm for 10 mins and supernatant transferred to another new tube. Ice-cold isopropanol (700 µl) was added, gently mixed and stored in a freezer (-20°C) for 1 hr to precipitate the DNA and then centrifuged at 12,000 rpm for 10 mins. The supernant was carefully discarded; tube was drained on clean paper towel for 1 hour.

The DNA pellet was washed twice in 100 µl cold 70% ethanol for 20 mins and air dry completely. Sixty microlitre (60 µl) of sterile distilled water was added to the pellet, followed by 2 ul of 10 ng/ml RNase. The final incubation of the solution was done for 40 mins at 37°C with gentle mixing at 10 mins intervals. The DNA was stored in -20°C.

Method 2: Zymo Research Plant/Seed DNA MiniPrep Kit (ZR Kit) was used, according to the manufacturer's protocol. Fresh tomato leaf of 150 mg was added to a ZR BashingBead™ Lysis Tube and 750 µl Lysis Solution added. Disruption of the cells was done at high speed for 10 min. The Lysis Tube was then centrifuged in a microcentrifuge at 10,000 x g for 1 min. About 400 µl supernatant was transferred to Zymo-Spin™ IV Spin Filter (orange top) in a Collection tube and centrifuged at 7,000 rpm (≈ 7,000 x g) for 1 min.

Thereafter, 1,200 µl of Plant/Seed DNA Binding Buffer (diluted with beta-mercaptoethanol to a final solution of 0.5% v/v) was added to the filtrate in the collection tube and mixed with micropipette. From the Collection tube, 800 µl of the mixture was transferred to Zymo-Spin™ IIC Column in a Collection tube and centrifuged at 10,000 x g for 1 min.

The flow through was discarded and 200 µl DNA Pre-Wash Buffer was added to the Zymo-Spin™ IIC Column in a new Collection tube and centrifuged at 10,000 x g for 1 min. Plant/Seed DNA Wash Buffer, 500 µl, was added to the Zymo-Spin™ IIC Column and centrifuged at 10,000 x g for 1 min. Zymo-SpinTM IIC Column was transferred to clean 1.5 ml microcentrfuge tube and 100 µl DNA Elution Buffer was added directly to the column matrix and centrifuged at 10,000 x g for 30 sec to elute the DNA. The eluted DNA from was transferred to a centrifuged Zymo-spin™ IV-HRC Spin Filter (green top) in a clean 1.5 ml microcentrifuge tube and centrifuged at exactly 8,000 xg for 1 min.

Gel electrophoresis and spectrophotometry

Agarose gel (1%) was prepared, microwaved at 100°C for 3 mins. and allowed to cool. Five microlitre of ethidium bromide (EtBr) was added to 150 ml agarose gel, poured into the gel tank and allowed to solidify. Subsequently, 3 µl of loading dye was added to 5 µl each of the samples. Samples were loaded and allowed to run at 100V for 45 min before viewing on UV light source and photographed. The quality of the extracted samples was evaluated by the ratio of the absorbance at 260 and 280 nm.

Results

Table 1 shows the quantity and purity of the extracted genomic DNA from five tomato cultivars using two different extraction methods. In ZR Kit extraction, genomic DNA concentrations ranged from 24.42 to 43.11 ng/µl with highest concentration of 43.11 ng/µl in Pure-water cultivar and 24.42 ng/µl in F1-Mongal cultivar. It was observed that Kerewa had a unique DNA yield (1430.52 ng/µl) above other cultivars using Dellaporta et al. protocol; with Pure-water cultivar produced the least yield of 687.51 ng/µl. In all, the highest amount of DNA was obtained from Kerewa (1430.52 ng/µl) while on the other hand F1-Mongal cultivar produce the least amount of DNA (24.42 ng/µl). DNA purity ranged from 1.11 to 2.00 in both methods. DNA purity from all tomato samples, when Dellaporta et al.method was used, ranged from 1.73 to 2.00. The genomic DNA samples from different plant materials were determined on agarose gel (Plate 1A and B). The DNA bands appeared to be compact with negligible smearing indicating little degradation during isolation (Plate 1 A) but there were smeared bands of DNA in Plate 1B. Dellaporta et al. method presented highly resolved bands indicating good quality of DNA (Plate 1A).

Table 1: Comparison of genomic DNA quantity and purity extracted using two different extraction methods.

S/N	Cultivar Name	Age (week)	Amount of leaf sample (g)	Concentration (ng/µl)		Purity of DNA (A260/A280)	
				ZR Kit method	Dellaporta et al., method	ZR Kit method	Dellaporta et al., method
1	F1-Mongal	2	1.50	24.42	871.50	1.11	1.99
2	Pure-water	2	1.50	43.11	687.51	1.33	1.73
3	Santana	2	1.50	25.33	798.18	1.22	2.00
4	Kerewa	2	1.50	27.25	1430.52	1.24	1.99
5	Tyre-type	2	1.50	36.18	924.65	1.45	1.92

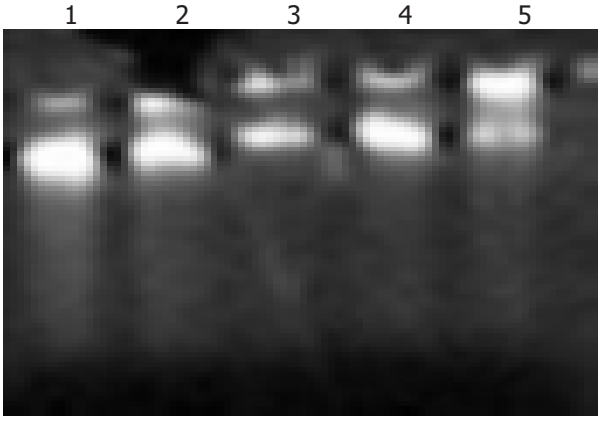

Plate 1 A: Agarose gel electrophoresis of the products obtained using a Dellaportal et al. method of DNA extraction from tomato leaf samples. Lane 1: F1-mongal; 2: Pure-water; 3: Santana; 4: Kerewa and 5: Tyre-type. DNA extracts were electrophoresed on 1% agarose gel at 100 V for 45 min, stained with ethidium bromide and visualized under UV illumination.

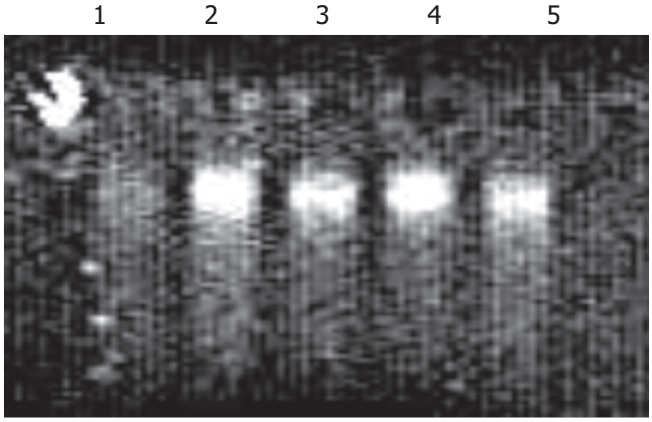

Plate 1 B: Agarose gel electrophoresis of the products obtained using Zymo Research Plant/Seed DNA MiniPrep Kit methods of DNA extraction from tomato leaf samples. Lane 1: F1-mongal; 2: Pure-water; 3: Santana; 4: Kerewa and 5: Tyre-type. DNA extracts were electrophoresed on 1% agarose gel at 100 V for 45 min, stained with ethidium bromide and visualized under UV illumination.

Discussion

Three contaminants associated with plant DNA can interfere PCR reactions, for example polyphenolic compounds, polysaccharides and RNA (Krishna et al., 2012) and these can hamper the isolation of good quality DNA (Arif et al., 2010). For molecular genetic analysis of crops, availability of high-quality cellular DNA is an essential precondition. When Dellaporta et al. method was used good quality DNA was extracted from Santana, F1-Mongal, Kerewa, Tyre-type and Pure-water cultivars which showed that contaminants were not present. This corroborated the report of Mirbahar et al. (2014) that good quality DNA isolation is necessary which is comparatively free from several contaminants found in plant cells. Pich and Schubert (1993) described DNA purity in the range of 1.6 - 1.7 the absence of contaminants. In this study, the results indicated that DNA extracted using ZR kit were contaminated. Mirbahar et al. (2014) further stated that extraction of intact, high molecular-weight DNA that can support PCR, genomic blot analysis, fingerprinting and other molecular analysis is not easy when the plant tissue is rich in polysaccharides, secondary metabolites or polyphenolics. The results of the PCR when commercial kit was used might be due to high amount of proteins in the plant which might bind firmly to nucleic acids during extraction of DNA which the commercial kit could not resolve. This was similar to the work of previous researchers who identified that high amount of proteins is present in many plant species naturally (Angeles et al., 2005) and other substances could bind firmly to nucleic acids during extraction of DNA which can interfere in DNA amplification (Ribeiro and Lovato, 2007).

To use methods common to different genotypes can help rapidly. In this study, different tomato cultivars DNA quantity and purity were used. Genomic DNA extracted from F1-Mongal, Pure-water, Santana, Kerewa and Tyre-type with Dellaporta et al. method of DNA isolation produced better results. The yield and purity from samples using ZR kit were lower, suggesting the presence of impurities. Agarose gel comparison of DNA also indicated the presence of degraded or smeared DNA in the samples from ZR kit as shown by the smeared appearance of the DNA on the gel. The results demonstrated that good yields of very pure, intact, high quality genomic DNA could be isolated from tomato leaves using the method of Dellaporta et. al.

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