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Research Article
Evaluation of Chelex 100 for DNA Extraction in Tomato Seedling (*Solanum lycopersicum*)

[1]Mehrnoush Aminisarteshnizi, [2]Matlawa Mohlabe and [2]Rebotile Lediga

[1]Department of Research Administration and Development, University of Limpopo, Turfloop Campus, Private Bag X1106, Sovenga 0727, South Africa
[2]Department of Pre-Clinical Sciences, University of Limpopo, Turfloop Campus, Private Bag X1106, Sovenga, 0727, South Africa

Abstract

Background and Objective: Tomato (*Solanum lycopersicum*) is a vegetable crop commonly used as a fresh vegetable or as a spice for food preparation. Extraction of genomic DNA with high quality for this critical vegetable is one of the basic needs of polymerase chain reaction. In modern research, PCR has found wide applications. **Materials and Methods:** This study had compared two different extraction DNA methods from tomato (*S. lycopersicum*). The DNA from the tomato was extracted using Chelex "method 1" (overnight incubation at 56°C) and "method 2" (Ten minutes incubation at 95°C) from the fresh leaves of the tomato. For this comparison, we used four samples of *S. lycopersicum* from South Africa during 2021. Quantitative and qualitative parameters were measured using a spectrophotometer. To confirm and evaluate the extracted DNA, the PCR reaction with primers for 28S was performed on all samples. **Results:** The results for the spectrophotometer showed that the highest quality of extracted DNA was in "method 2" (1.59-1.64). However, the protein (1.46-1.50 mg mL^{-1}) was detected in the tested samples through "method 1".The qualitative and quantitative tests for PCR reaction showed that the DNA extracted using "method 2" had better quality than "method 1". Amplification of samples with 28S primer showed higher concentration and purity of DNA extracted with "method 2". **Conclusion:** In conclusion, both methods worked, but method two showed better results regarding time and high-quality DNA.

Key words: DNA extraction, *Solanum lycopersicum*, spectrophotometer, PCR, amplification

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Corresponding Author: Mehrnoush Aminisarteshnizi, Department of Research Administration and Development, University of Limpopo, Turfloop Campus, Private Bag X1106, Sovenga 0727, South Africa

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Most of the studies base on plants are performed at the molecular level, which needs reliable and quick DNA extraction protocols. DNA extraction with high quality is a foundation for further study in the molecular field[1]. The various methods for genomic DNA created many DNA extraction methods[2]. However, some methods such as chloroform-based DNA extraction is not safe for the human. Additionally, chloroform-based DNA extraction needs the use of toxic chemicals, magnetic separation and silica-based DNA extraction incline to be expensive[1].

Pure and rapid extraction of DNA is a prerequisite for most advanced techniques such as genetic mapping, fingerprinting and marker-assisted selection. However, the extraction of high-quality DNA can be time-consuming, arduous and costly due to multiple steps[2]. The Chelex method has proven to be efficient in extracting DNA in PCR analyses in a wide range of experiments. Chelex procedures are simple, rapid, do not involve harmful organic solvents and do not require multiple transfers between tubes for most types of samples[1].

The quantity and quality of the extracted DNA particularly sample dependent. Furthermore, the chemical-physical composition affects DNA extraction. Several plant species, including the Solanaceae family, produce secondary metabolites like phenolic compounds, tannins, flavonoids and alkaloids, which present in the extract solution can interfere with DNA analysis and inhibit the PCR processing[3].

Tomato (*S. lycopersicum*) is a major vegetable crop commonly grown by farmers from South Africa[4]. Tomato is a critical and high-demand vegetable in Limpopo Province, South Africa, which genomic DNA is essential for genetic diversity and molecular analysis of this crop. The chelex method has been proven to be an optimistic method for DNA extraction in various organisms such as nematodes[5]. The previous result indicated that DNA extraction in a diverse group of nematodes such a free-living (*Butlerius butleri*) and plant-parasitic nematode (*Helicotylenchus*) could be done using the Chelex method[6]. The extracted DNA of nematode yielded a high quality with successful PCR processing for various DNA markers[5]. However, the Chelex method in different organisms needs to be adjusted regarding the timing of application for Chelex. Besides, in animal tissues, proteinase K needs to be used along with the Chelex[5,6], which is unnecessary for plant tissues.

Hence, this study aimed to evaluate the Chelex efficiency for DNA extraction in tomatoes with an overnight incubation at 56°C and 2) to evaluate the Chelex efficiency for DNA extraction in tomatoes with ten minutes incubation at 56°C.

MATERIALS AND METHODS

Sample collection: Four samples of *S. Lycopersicum* were collected from the commercial Floradade seedling in Polokwane (23°52'24.695"S, 29°30'44.294"E) in 2021. All samples transfer to the molecular lab for DNA extraction and molecular analysis.

Molecular analysis: In "method 1", DNA extraction was done using the Chelex method[5]. Specimens of *S. lycopersicum* were hand-picked and transferred to a 1.5 mL Eppendorf tube containing 20 µL double distilled water. The tomato leaves in the tube were crushed with the tip of a fine needle and vortexed. Sixty microliters of 20% Chelex® were added to each microcentrifuge tube containing the crushed tomatoes and mixed. The tubes were incubated at 56°C overnight for 12 hrs, finally, spined for 2 min at 16000 rpm[6]. The PCR product was stored at -20°C.

The "method 2" for DNA extraction was done using the modified Chelex method[5]. Specimens of *S. lycopersicum* were hand-picked and transferred to a 1.5 mL Eppendorf tube containing 20 µL double distilled water. The tomato leaves in the tube were crushed with the tip of a fine needle and vortexed. Sixty microliters of 20% Chelex-100® were added to each microcentrifuge tube containing the crushed tomatoes and mixed. The tubes were incubated at 95°C for 10 min. The mixture was vortexed for 10-30 s. Then the tubes were centrifuged at 16000 rpm for 1 min and the supernatant was used as a template for PCR. Each method was repeated with four replicates. Thus, the experiments were performed twice.

Spectrophotometric analyses of DNA: Thermo Scientific NanoDrop™ One Spectrophotometer (Thermo Scientific, Germany) was used to determine sample concentration, purity and absorbance ratio at 260-280 nm (A260/A230 ratio). These were measured using 1 µL of each sample. Thus, each sample's measurements were repeated three times.

PCR amplification: The 28S ribosomal DNA is a commonly used DNA marker in DNA barcoding analysis recommended in plant DNA barcodes. For Polymerase Chain Reaction (PCR) analysis, the forward and reverse primers, D2A (5'-ACAAGTACCGTGAGGGAAAGTTG–3'), D3B (5'-TCGGAAGGAACCAGCTACTA–3')[7] was used for partial amplification of the 28S rDNA. PCR was conducted with 5 µL of the DNA template, 12.5 µL of 2X PCR Master Mix Red (Promega, USA) for the South African specimens, 1 µL of each primer (10 pmol μL^{-1}) and ddH_2O for a final volume of 30 µL. The PCR processing was done using an Eppendorf master

cycler gradient (Eppendorf, Hamburg, Germany), with the following program: 94°C for 3 min at, next 37 cycles of denaturation for 45 s at 94°C; 56°C annealing temperatures for 28S rDNA; extension for 45 s to 1 min at 72°C and finally an extension step of 6 min at 72°C followed by a temperature on hold at 4°C. After DNA amplification, 4 µL of product from each tube was loaded on a 1% agarose gel in TBE buffer (40 mM Tris, 40 mM boric acid and one mM EDTA) to evaluate the DNA bands. The PCR products were evaluated using Red Gel dye and visualized and photographed using a UV transilluminator[8].

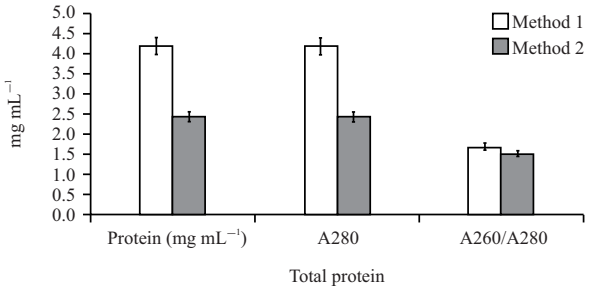

Fig. 1: Total protein obtained for all tomato sample extracts using methods 1 and 2

RESULTS AND DISCUSSION

DNA quality and quantity assessment: The quality of extracted DNA sample was evaluated using a Nanodrop instrument. It was observed that high-quality DNA ranged between 1.8 and 2.0 at A260/280. In this study ratio of 260/280 was found to be in a range of 1.46-1.64. The DNA extracted must be free of contaminating substances, such as polysaccharides and phenols. The extraction and purification of high-quality DNA are generally tricky. The presence of these compounds affects the quality and quantity of isolated DNA, rendering the sample non-amplifiable[2]. The "method 2" produced DNA samples with purity ratios in a range of 1.59-1.64, whereas the purity ratio of samples extracted by "method 1" was between 1.46-1.50 in Table 1.

For the polymerase chain reactions, approximately 100 ng μL^{-1} of DNA concentration is necessary for the PCR product to be seen after 30 cycles. The DNA concentration for "method 2" was found to be in a range of 136-214 ng μL^{-1}, whereas for "method 1", it was in a range of 87-168 ng μL^{-1}.

The Nanodrop device measured the amount of protein extracted from the samples in Fig. 1. The results showed that the amount of protein in "method 1" and"method 2" was different. Furthermore, it was observed that a higher total protein in "method 1" compared with "method 2". These results could explain the reason for the low quality of the "method 1".

PCR amplification for DNA detection: The extracted DNA of methods one and two were used for DNA extraction of a fresh sample of *S. lycopersicum* and detected using the 28S rDNA primer. The rDNA-28S fragment was produced by DNA amplification following "method 1" and "method 2". The fragment size was approximately 680 base pairs in Fig. 2, consistent with the expected result. The result indicated a good band in the PCR products. The amplified bands obtained from "method 2" extraction of tomato samples were neat and clear, compere to "method 1".

The Nanodrop absorbance profile is helpful for the detection of contaminants such as polysaccharides, salts and proteins, which can interfere with and inhibit DNA sequencing. The ratio of 1.8 in 260/280 nm indicated that the extracted DNA had high quality with the absence of proteins and phenols. A purity ratio higher than 1.9 indicated the presence of RNA in the extracted DNA sample, however, it did not have more than 1.9 value. The ratio of <1.7 in some samples of DNA extracted by the "method 1" suggests the presence of higher total proteins in those samples. These differences could be explained by the ability of some of the procedures to eliminate contaminating molecules. Liu *et al.*[9] reported that DNA quality was evaluated by polymerase chain reaction. The results showed that genomic DNA extracted using the Chelex-100 method were better than using the CTAB method.

Chelex has been investigated on the nematodes with high-quality PCR products[10,11,12]. However, in plants, DNA extraction needs no proteinase K, which is an advantage. Turan *et al.*[13] used the Chelex method for extracting DNA for *Venturia inaequalis* spores. Chelex resin was evaluated and compared with a well-established DNA extraction method utilizing CTAB. They reported that the quality of DNA samples isolated using the Chelex method was better than those extracted using CTAB. HwangBo *et al.*[14] used Chelex for extracting DNA in some plants such as tomatoes. The PCR analysis showed successfully amplify transgenes. The result obtained in this study agrees with the result obtained by several studies in this field. Singh *et al.*[15] studied a method for improving the quality of genomic DNA obtained from minute quantities of tissue and blood samples using Chelex 100 resin. They found the Chelex method was non-toxic, easily available

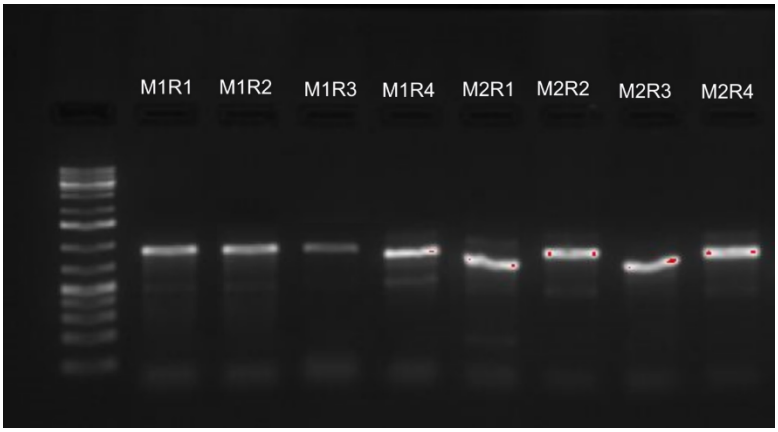

Fig. 2: Agarose gel electrophoresis of rDNA-28S primers in "method 1" (M1R1-M1R4) and "method 2" (M2R1-M2R4) for tomato collected in Limpopo province, South Africa

Table 1: Mean DNA obtained for all sample extracts using method one and two (Mean±SE)

Samples	Nucleic acid (ng μL^{-1})	260/280	260/230	A260	A280
M1R1	168±22	1.50±0.01	0.59±0.02	3.36	2.23
M1R2	87±15	1.46±0.02	0.46±0.01	1.74	1.19
M1R3	101±19	1.47±0.01	0.51±0.01	1.92	1.3
M1R4	152±21	1.49±0.03	0.57±0.02	3.33	2.23
M2R1	214.49±23	1.64±0.01	0.68±0.02	4.29	2.60
M2R2	136.5±22	1.59±0.02	0.64±0.01	2.73	1.71
M2R3	201±25	1.61±0.01	0.67±0.02	4.3	2.59
M2R4	185±24	1.6±0.02	0.62±0.01	2.69	1.68

M1: Method 1, M2: Method 2

and inexpensive reagents, as well as minimal amounts of blood or tissue samples for the DNA extraction process. Sajiba *et al.*[16] studied a simple, efficient and rapid Chelex method for good quality DNA extraction from rice grains. They reported this method reproducibly extracts DNA with good purity indices and requires only a few steps. Therefore, it was tried to provide a better protocol for DNA extraction by the Chelex method in this study. Generally, in different DNA extraction protocols, polyphenolic residues mainly inhibit DNA polymerase activity during PCR analyses[17]. In the present study, only Chelex was used to extract DNA. The PCR analyses showed that the DNA samples prepared by both methods could be successfully used for PCR amplification with an rDNA primer. Thus, these methods could be used for genetic diversity and phylogenetic purposes.

CONCLUSION

The use of Chelex to extract DNA is prevalent among researchers. In this study, two methods for extracting DNA from *S. lycopersicum* were used. Both methods worked, but the quality of "method 2" was much better than the "method1". High quality of DNA is required for PCR and sequencing. Therefore, it must be free of contamination from protein, RNA, or polysaccharides. Furthermore, among the two methods, "method 1" needs more time (12 hrs) than "method 2" (10 min), which causes extraction of the DNA with higher total protein. Additionally, the low cost of Chelex, which allows us to use it for many DNA extractions, creates an excellent option for molecular research in plant genetics and phylogenetic studies. Therefore, Chelex is highly recommended for DNA extraction from plant sources especially in the family Solanaceae.

SIGNIFICANCE STATEMENT

A high-quality DNA is essential for molecular analysis in various plant studies. The result of the present study using Chelex suggests that this method helps to obtain high-quality DNA from the plant source. As tomato is the high demand crop in South Africa, various molecular studies are needed aiming for the best varieties and yield increasing. This study

will help the researchers to uncover the critical areas of the different protocols that many researchers were not able to explore. Thus, a new theory on these protocols for extracting DNA with Chelex may be arrived at.

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