Open Access

SCHOLARENA

Journal of Advancements in Plant Science

RESEARCH ARTICLE

ISSN: 2639-1368

An Improved Extraction that Increases DNA Yield in Chile Pepper *(Capsicum* Sp.*)*

Ortega FA[1], Hayano-Kanashiro C[2] and Bosland PW[1*]

[1]*Department of Plant and Environmental Sciences, New Mexico State University, Las Cruces, New Mexico, United States of America*
[2]*Department of Scientific and Technological Research DICTUS, Universidad de Sonora, Sonora, Mexico*

[*]**Corresponding author:** Bosland PW, Department of Plant and Environmental Sciences, New Mexico State University, Las Cruces, New Mexico, 88003 United States of America, Tel: +5756465171, E-mail: pbosland@nmsu.edu

Citation: Ortega FA, Hayano-Kanashiro C, Bosland PW (2019) An Improved Extraction that Increases DNA Yield in Chile Pepper (Capsicum Sp.). J Adv Plant Sci 2: 106

Article history: Received: 16 April 2019, **Accepted:** 10 June 2019, **Published:** 12 June 2019

Abstract

Next generation sequencing requires large amounts of high quality genomic DNA. This constraint requires researchers to customize DNA extraction methods for maximum efficiency. For a genotyping-by-sequencing project, we found that extracting DNA from leaf tissue of the *Capsicum* species, using a mortar and pestle homogenization step and following manufacturers procedures from the DNeasy Mini Plant Kit (QIAGEN, Germany), produced insufficient amounts of genomic DNA. The protocol was altered to address this extraction limitation. While it was initially suspected that secondary metabolite content may have been complicating the extraction, we found instead that simply switching the homogenization method to a bead mill more dramatically increased the quantity of DNA retrieved, underscoring the importance of thorough tissue homogenization for extracting large concentrations of genomic DNA.

Keywords: Bead Mill; DNA Extraction; Homogenization

Introduction

DNA isolation represents the basic and probably most important procedure for plant genetics programs, although sometimes extraction from certain species and tissues can present obstacles that can hinder downstream applications, and ultimately project advancement. The challenge of extracting from recalcitrant tissue can be compounded depending on the intended analytical purpose. For genotyping by sequencing (GBS) projects, it is critical to use high molecular weight DNA that is free from enzyme-inhibiting contaminants to ensure reliable genotyping results [1,2]. Meeting such purity and yield requirements sometimes necessitate the alteration of a DNA extraction protocol to suit the tissue that is being used.

Commonly reported problems with plant DNA extraction involve sample contamination due to the presence of polysaccharides, phenolic compounds, and other secondary metabolites in the tissue that limit use in downstream applications [3-5]. Such plant contaminants interact with nucleic acids, and the co-extraction of these contaminants along with DNA can cause interference with polymerases, restriction nucleases, and ligases [5,6]. Commercial kits, like the QIAGEN DNeasy Plant kit, facilitate quick extractions that are meant to effectively isolate DNA from inhibitory secondary metabolites by employing silica columns to bind the DNA while the buffer washes over it, separating the DNA from carbohydrates, polyphenols, and other plant metabolites [7, 8]. Although, with plant tissues that have exceptionally high concentrations of polyphenolics like sunflower (*Helianthus annuus*) and cotton (*Gossypium hirsutum*), it can still be challenging to extract high quantity and quality DNA, even when using commercial kits [9]. While there is very little information about secondary metabolite content in pepper (*Capsicum* sp.) specifically, it is a perennial plant and perennial plants possess impurities such as Terpenes, Polyphenols, and Polysaccharides that could be co-extracted [10].

A different but perhaps more fundamental challenge faced in DNA extraction is incomplete tissue homogenization. Tissue homogenization and cell lysis are the first steps in all molecular biology and molecular diagnostic techniques, necessary to disrupt the cells and release the genetic material [11]. One common and inexpensive tissue grinding method is to use a mortar and pestle to crush the tissue in the presence of liquid nitrogen. Individually grinding each sample before extraction can be time consuming, but more importantly, it can produce samples that are inconsistently, and sometimes ineffectively, homogenized [12]. If tissue has been ineffectively homogenized, the amount of DNA in the starting materials will be limited, which ultimately affects the yield and purity of the final product [13]. There are multiple methods for disrupting tissue, but bead mill homogenization, where buffer, tissue, and milling beads are combined in a container and ground through rapid oscillation, seems to be the method with the most potential for increasing DNA yield [14].

Difficulties were encountered during the DNA extraction from the leaf tissue of *Capsicum* species for a genotyping by sequencing (GBS) project. Using the QIAGEN DNeasy Plant Mini Kit (QIAGEN, Germany), the manufacturer's protocol with a mortar and pestle grinding step produced insufficient amounts of genomic DNA (producing from 2.0 to 10.0 ng/µL). Low yield prompted modifications to be made to the DNeasy Plant Mini Kit protocol. Modifications were made in response to two common problems faced in plant DNA extraction: co-extraction of secondary compounds, and poor tissue homogenization that were addressed by doubling the volume of lysis buffer and switching homogenization with a mortar and pestle for homogenization with a bead mill, respectively.

Materials and Methods

To test the efficacy of different extraction protocols, four (100 mg per sample) young leaf tissue samples were taken from each of three *Capsicum* species: Keystone (*C. annuum*), Tabacco (*C. frutescens*), and Orange Habanero (*C. chinense*). The DNeasy Plant Mini Kit and protocol were used with modifications being made to the first step. This meant changing the method of homogenization and/or the amount of AP1 buffer (lysis buffer) added during homogenization. Four methods were tested: 1) addition of 400 µL of Buffer AP1 ground with a mortar and pestle in liquid nitrogen; 2) addition of 800 µL Buffer AP1 ground with a mortar and pestle in liquid nitrogen; 3) addition of 400 µL Buffer AP1 and homogenized with a bead mill; 4) addition of 800 µL of Buffer AP1 and homogenized with a bead mill. Tissue collected from the same plants was used in each method.

Dneasy Plant Mini Kit Protocol

Tissue was disrupted and 400 µL Buffer AP1 and 4 µL RNase A were added. The AP1 and RNase A were mixed by vortexing, and the samples were left to incubate for 10 min at 65 °C on a heat block. During incubation, the samples were inverted every two minutes. Then samples were retrieved from the heat block, 130 µL Buffer P3 was added, the tubes were inverted, and the samples were placed for 5 min on ice. After the incubation, they were centrifuged for 5 min at 14,000 rpm and the supernatant was collected into a QIA shredder spin column. The column was centrifuged for 2 min at 14,000 rpm. The flow through from the column was poured into a new tube where the volume was measured. This volume was multiplied by 1.5 to find the volume of Buffer AW1 to add to each tube. The lysate and the buffer were mixed by pipetting and 650 µL of the mixture were transferred into a DNeasy Mini spin column and centrifuged for 1 min at 8,000 rpm. Flow through from each column was discarded, and the column was refilled with the remaining mixture and centrifuged again under the same parameters. The spin column was moved to a clean tube where 500 µL of Buffer AW2 was added and centrifuged again for 1 min at 8,000 rpm. Flow through after the spin was discarded and another 500 µL Buffer AW2 was added to the column. This time the column was centrifuged for 2 min at 14,000 rpm. The spin column was then moved to the final 1.5 mL microcentrifuge tube where 50 µL of nuclease free water were added directly to the membrane and incubated for 5 min at room temperature and centrifuged for 1 min at 8,000 rpm. This final step was repeated. Column was then discarded, and the samples stored at -20 °C.

1) Recommended AP1 and Mortar and Pestle Homogenization

This method is the same as the Dneasy Plant Mini Kit Protocol, with the addition of the recommended 400 µL Buffer AP1 followed by disruption using a mortar and pestle in the presence of liquid nitrogen.

2) Doubled Buffer AP1 and Mortar and Pestle Homogenization

This protocol is the same as the Dneasy Plant Mini Kit Protocol, with the exception of 800 µL Buffer AP1 being added to each sample before homogenization with a mortar and pestle, followed by the addition of 8 µL Rnase A. The extra volume means that more AW1 is used in a later step and subsequently the Mini Spin Column is filled with the lysate/AW1 mixture and centrifuged for 1 min at 8,000 rpm three times instead of two.

3) Recommended AP1 and Bead Mill Homogenization

Tissue was collected in 2.0 mL screw-cap tubes with five 2.5 mm Zirconia/Silica beads inside. Prior to tissue collection, with the beads inside the tube, all tubes were sterilized. Before homogenization, 400 µL of Buffer AP1 was added to each tube, and the tissue was lysed with a Precellys 24 Homogenizer (Bertin Instruments, France). The bead mill was run at 6,500 rpm for three rounds of shaking lasting 30 seconds each. After homogenization, the tubes were centrifuged for 5 min at 14,000 rpm and the supernatant was transferred to a 1.5 mL microcentrifuge tube, leaving the beads behind. Then 4 µL of RNase was added to each sample. After this step, the DNeasy Plant Mini Kit protocol was followed.

4) Doubled AP1 and Bead Mill Homogenization

Tissue was collected as described in protocol 3, Bead Mill Homogenization. Prior to use of the Precellys 24 Homogenizer, 800 µL of Buffer AP1 was added to each tube. Homogenization took place as described in Modified Protocol 2, and 8 µL of RNase was added to each sample. After homogenization the tubes were centrifuged for 5 min at 14,000 rpm and the supernatant was transferred to a 1.5 mL micro centrifuge tube. Then, extraction proceeded according to the DNeasy Plant Mini Kit protocol.

Concentration and absorbance were measured on a NanoDrop 2000 at 260nm and 280nm (Thermo Scientific) using 2 µL DNA. The ratio A_{260}/A_{280} should be between 1.80 and 2 for pure DNA samples [15].

Results

DNA quality and yield assessment

Each extracted DNA sample from leaves of the three *Capsicum* species was verified using a Nanodrop spectrophotometer. The purity of DNA samples was inferred from recorded absorbance ratios.

The range concentrations for the four protocols tested on three samples was between 1.6 and 878.2 ng/μL (Table 1). The average concentrations for each method were as follows: 1) 1xAP1 Mortar and Pestle: 7.4 ng/μL, 2) 2xAP1 Mortar and Pestle: 15.8 ng/μL, 3) 1xAP1 and Bead Mill Homogenization: 741.9 ng/μL, and finally 4) 2xAP1 and Bead Mill Homogenization: 541.4 ng/μL (Table 1). When comparing DNA concentration according to volume of AP1 regardless of homogenization method, the average concentration for a sample extracted with 1xAP1 was 374.6 ng/μL, and the average concentration for a sample extracted with 2xAP1 was 278.6 ng/μL. However, when grouped according to homogenization method regardless of AP1 volume, the average concentration for mortar and pestle homogenized samples was 11.6 ng/μL, and the average concentration for bead mill homogenized samples was 641.7 ng/μL. Additionally, samples lysed with a bead mill had more consistent absorbance values that were closer to the ideal ratio $A_{260/280}$ at 1.80 and the ratio $A_{260/230}$ at 2.00-2.2 [15] than those lysed with a mortar and pestle (Table 1). This suggests that the greatest increase in quality and quantity was due to changing the homogenization method rather than the increase of buffer.

Discussion

Next generation sequencing projects require large quantities of high quality genomic DNA to ensure reliable production of genotyping data. Obtaining this DNA from plant tissue comes with the challenge of separating DNA from secondary metabolites that can inhibit enzymatic reactions [1-3]. Carrying out DNA extraction from *Capsicum* leaf tissue using the DNeasy Plant Mini Kit (QIAGEN) with a mortar and pestle grinding step produced low yield and purity of genomic DNA. Two modifications to the protocol addressed two common problems with DNA extraction. These were: doubling the amount of AP1 (lysis buffer) to aide in separation of nucleic acids from secondary metabolites, and employing a bead mill system to ensure thorough and uniform homogenization.

Aside from a study reporting the presence of capsaicinoids in the leaves and stems during fruiting [16] and a recent attempt to identify phytochemical resistance to thrips (*Thrips* sp.) [17], very little has been reported about the content of secondary metabolites in the leaves of *Capsicum* compared to other higher plant species. The addition of extra lysis buffer was meant to maximize protection from DNases and increase the content of salt and polyvinylpyrrolidone to help separate the DNA from any polysaccharides and polyphenolics that may be in present in the sample [18, 19]. In this study, a slight increase in DNA concentration and quality was observed when comparing the mortar and pestle 1xAP1 treatment with the mortar and pestle 2xAP1 treatment (Table 1). This slight increase suggests, to some degree, that protein and polysaccharide content in the leaves could contribute to poor yield of DNA. However, the increase was not observed when comparing the bead mill homogenized 1xAP1 treatment to the bead mill homogenized 2xAP1 treatment. Instead, the average for the bead mill homogenized 2xAP1 was lower than its 1xAP1 counterpart, which could mean that the additional buffer may have restricted bead movement in the tube, impeding complete homogenization.

When compared to the mortar and pestle homogenized samples, samples homogenized with the bead mill increased the concentration by approximately 50-fold. Low $A_{260/230}$ ratios can mean that there are solvent residues or other contaminants present in the final product that absorb at the 230 nm peak. In our data, the bead mill-produced samples had a consistently higher $A_{260/230}$ ratio than those lysed by a mortar and pestle (Table 1). An absorbance ratio of 1.8 or greater indicates that the sample is pure or close to pure DNA [15], and all the bead mill preparations had an $A_{260/280}$ of 1.8, while the mortar and pestle ground samples had an $A_{260/280}$ that ranged from 1.54 to 2.21 (Table 1). This suggests that insufficient homogenization was the limiting step in extraction of high quality DNA from Capscium species.

#	Sample	Concentration (ng/μL)	Treatment	Homogenization Method	$A_{260/280}$	$A_{260/230}$
1	*C. frutescens*	1.6	1xAP1	Mortar & Pestle	1.99	.72
2	*C. chinense*	13.3	1xAP1	Mortar & Pestle	1.54	.64
3	*C. annuum*	7.3	1xAP1	Mortar & Pestle	2.21	.65
4	*C. frutescens*	19.4	2xAP1	Mortar & Pestle	1.71	.77
5	*C. chinense*	14.2	2xAP1	Mortar & Pestle	1.60	.96
6	*C. annuum*	13.8	2xAP1	Mortar & Pestle	1.89	1.86
7	*C. frutescens*	878.2	1xAP1	Bead Mill	1.85	2.30
8	*C. chinense*	823.6	1xAP1	Bead Mill	1.86	2.25
9	*C. annuum*	524.0	1xAP1	Bead Mill	1.83	2.28
10	*C. frutescens*	659.4	2xAP1	Bead Mill	1.84	2.25
11	*C. chinense*	768.4	2xAP1	Bead Mill	1.83	2.25
12	*C. annuum*	196.4	2xAP1	Bead Mill	1.85	2.23

Table 1: Extraction of high quality DNA from *Capscium species*

Conclusion

Methods to address contaminants like polysaccharides, phenolic compounds, and secondary metabolites are prevalent in plant DNA extraction literature. This is understandable considering the broad array of plants and tissues that would be challenging to extract from without the resource of modified DNA extraction methods. However, the predominance of these issues can obscure a discussion about simpler, but critical, DNA extraction steps. We found that switching from a mortar and pestle to a bead mill homogenization method dramatically and consistently increased the DNA quality and quantity retrieved from the leaves of *Capsicum* species. These results emphasize the importance of thorough tissue homogenization as a critical step that limits DNA yield and should not be overlooked.

Acknowledgments

Funding for this project was provided by the FAO Norman E. Borlaug International Agriculture Science and Technology Fellowship for C.H-K, and the New Mexico Chile Association.

References

1. Healey A, Furtado A, Cooper T, Henry RJ (2014) Protocol: a simple method for extracting next-generation sequencing quality genomic DNA from recalcitrant plant species. Plant Methods 10: 21.

2. Pompanon F, Bonin A, Bellemain E, Taberlet P (2005) Genotyping errors: causes, consequences and solutions. Nat Rev Genet 6: 847-59.

3. Scott KD, Playford J (1996) DNA extraction technique for PCR in rain forest plant species. Biotechniques 20: 974-77.

4. Shepherd M, Cross MJ, Stokoe RL, Scott LJ, Jones ME (2012) High-throughput DNA extraction from forest trees. Plant Mol Biol Reporter 20: 425.

5. Hiesinger M, Löffert D, Ritt C, Oelmüller U (2001) The effects of phenol on nucleic acid preparation and downstream applications. QIAGENews 5: 23-6.

6. Azmat MA, Khan IA, Cheema HMN, Rajwana IA, Khan AS, et al. (2012) Extraction of DNA suitable for PCR applications from mature leaves of Mangifera indica L. J Zhejiang Univ Sci B 13: 239-43.

7. Yang DY, Eng B, Waye JS, Dudar CJ, Saunders SR (1998) Technical note: improved DNA extraction from ancient bones using silica-based spin columns. Am J Phys Anthropol 105: 539-43.

8. Abdel-Latif A, Osman G (2017) Comparison of three genomic DNA extraction methods to obtain high DNA quality from maize. Plant Methods 13:1.

9. Horne EC, Kumpatla SP, Patterson KA, Gupta M, Thomson SA (2004) Improved High-Throughput Sunflower and Cotton DNA Extraction and PCR Fidelity. Plant Mol Biol Reporter 22: 83-4.

10. Pagare S, Bhatia M, Tripathi N, Bansal YK (2015) Secondary metabolites of plants and their role: overview. Current Trends Biotechnol Pharm 9: 293-304.

11. Kido H, Micic M, Smith D, Zoval J, Norton J, et al. (2007) A novel, compact disk-like centrifugal microfluidics system for cell lysis and sample homogenization. Colloids Surf B Biointerfaces 58: 44-51.

12. Eggermont K, Goderis IJ, Broekaert WF (1996) High-throughput RNA extraction from plant samples based on homogenisation by reciprocal shaking in the presence of a mixture of sand and glass beads. Plant Mol Biol Reporter 14: 273-9.

13. Csaikl, U, Bastian H, Brettschneider R, Gauch S, Meir A, et al. (1998) Comparative analysis of different DNA extraction protocols: a fast, universal maxi-preparation of high quality plant DNA for genetic evaluation and phylogenetic studies. Plant Mol Biol Reporter 16: 69-86.

14. Miller DN, Bryant JE, Madsen EL, Ghiorse WC (1999) Evaluation and optimization of DNA extraction and purification procedures for soil and sediment samples. Appl Environ Microbiol 65: 4715-24.

15. Thermo Fisher Scientific (2008) 260/280 and 260/230 Ratios. T042-Technical Bulletin Nano Drop Spectrophotometers.

16. Estrada B, Bernal MA, Diaz J, Pomar F, Merino F (2002) Capsaicinoids in vegetative organs of Capsicum annuum L. in relation to fruiting. J Agric Food Chem 50: 1188-91.

17. Maharijaya A, Vosman B, Pelgrom K, Wahyuni Y, de Vos RCH, et al. (2018) Genetic variation in phytochemicals in leaves of pepper (Capsicum) in relation to thrips resistance. Arthropod-Plant Interactions 13 : 1-9.

18. El-Ashram S, Al Nasr I, Suo X (2016) Nucleic acid protocols: Extraction and optimization. Biotechnol Rep 12: 33-9.

19. Fang G, Hammar S, Grumet R (1992) A quick and inexpensive method for removing polysaccharides from plant genome DNA. Biotechniques 13: 52-4.