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RESEARCH NOTE

DNA extraction protocol for tomato and arabidopsis plants using Edwards' buffer

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Abstract

The most effective barriers preventing the collection of pure DNA from plant samples is the presence of the secondary products. Most available DNA extraction protocols were prepared to eliminate this obstruction. As a first step, Edwards' buffer was used to isolate DNA from the leaves of tomato (*Lycopersicon esculentum* Mill.), which is known to contain more secondary metabolites especially polyphenol, comparing to Arabidopsis (*Arabidopsis thaliana* L.). This protocol has been intensively and successfully used in Arabidopsis but not for tomatoes. Results indicated that the collected DNA; without cold centrifugation, from Arabidopsis appeared as normal white jelly pellets with high purity ratio (1.93±0.072), while the DNA pellets from tomato samples showed dark color and with less purity (1.73±0.048). This difference in DNA purity was highly significant. Also, there was significant difference in DNA concentration between the two plant species; 316.38±38.0 ng/µl for Arabidopsis and 71±38.78 ng/µl for tomato. These results indicate that Edwards' buffer method works well with Arabidopsis but not necessarily with Tomato unless some modifications are applied to the protocol. That is to eliminate secondary products from samples, with keeping its advantages as a very simple, safe, and not time-consuming way to extract genomic DNA suitable for PCR and some other tests needed in plant molecular biology.

Key words: Arabidopsis, tomato, Edward's buffer, DNA, polyphenol

Highly qualitative DNA is required and essential for many molecular biology techniques. The extraction of DNA from plant tissues is often time-consuming, laborious, needs large amounts of plant tissue and involves many chemicals (Fulton *et al.*, 1995). In general, plants contain many secondary metabolites which interfere with extracted DNA and affect DNA purity and quality. Scientists have developed different extraction protocols to overcome these obstructions. Tomato, like many other plants, contains high levels of tannins and other polyphenolic compounds. Several DNA extraction protocols for isolating genomic DNA from different plant sources have been described. Even though new protocols rose up to extract DNA (Vilanova *et al.*, 2020), the Cetyltrimethyl Ammonium Bromide (CTAB) as an older protocol and its modifications still extensively used in various laboratories (Semagn, 2014) despite its time consuming and chemicals involvement (Cheng *et al.*, 2003).

Other traditional DNA extraction methods help to remove some contaminants but require large amounts of plant tissue (Jobes *et al.,* 1995) which is time consuming and may hamper DNA quality. An alternative protocol for genomic DNA extraction from fresh and dry plant leaves that is suitable for PCR-based genetic analysis (Chabi *et al.,* 2015). In this method, SDS and salt with high concentrations were used to isolate DNA from cashew trees without the use of hazardous chemicals. On the other hand, Peterson et al. (Peterson, *et al.,* 1997) were able to prevent DNA - polyphenol oxidation in tomato samples but they had to use more chemicals and spend extra time for DNA preparation.

Edwards' buffer (Edwards *et al.,* 1991) has been successfully used to extract pure DNA suitable for PCR analysis in Arabidopsis (Kasajima *et al.,* 2004; Elhaj, 2009 and 2021) and Brassica plants (Edwards *et al.,* 1991). This method does not need to use phenol and chloroform. Also, DNA can be extracted within very short time using inexpensive chemicals. Edwards' buffer method was modified by Amani et al. for DNA extraction from Canola and Tobacco plants, but this modification required more time and extra chemicals were used; chloroform:iso-amyl alcohol (Amani *et al.,* 2011). For PCR-based identification and characterization of Arabidopsis mutant lines, another modification of Edwards' method was used by Berendzen et al. (Berendzen *et al.,* 2005). DNA was collected using small amount of plant tissue and was with sufficient quantity and quality valid for PCR analysis for insertion mutants screening. Just recent modification of Edwards' method been published by Hu and Lagarias (Hu and Lagarias, 2020). The modification represents a tiny change in the way isopropanol was added. It is added directly to the crude sample, which has led to save time and reduce the cross contamination while yielding high quality DNA suitable for PCR analysis. Also, it is environmentally friendly by saving the number of tubes needed which helps to eliminate mislabelling of tubes especially when preparing many samples. Moreover, this method developed by Hu and Lagarias proved to be superior to the Kasajima's method which is known the most rapid protocol available (Kasajima *et al.,* 2004). Hence, by considering all above points the study was planned to develop DNA extraction protocol which can be subsequently used across labs in DNA extraction. Here we used two plants to develop DNA extraction protocol from tomato and Arabidopsis using Edwards' buffer.

Arabidopsis (*Arabidopsis thaliana* L.) Columbia 8 background and tomato (*Lycopersicon esculentum* Mill.) variety Rio Grande seeds were sown at appropriate conditions for germination. Plants were then grown at 23±1°c, 55-75 % relative humidity, and 135 µmol/ m-2/ s-1 light intensity. After three to four weeks from germination for Arabidopsis and tomatoes respectively, samples of 5-6 leaflets or leaves from tomato and Arabidopsis were collected in sterile Eppendorf tubes and kept at -20c° for later extraction. For cell DNA isolation, 400µl of autoclaved Edwards' buffer (Edwards et al. 1991) (200mM Tris-Cl pH 7.5, 250 mM NaCl, 25 mM EDTA pH 8.0, 0.5% w/v SDS) were added to the samples. Then, DNA was collected using 300 µl isopropanol without cold centrifugation. The DNA pellets were then dissolved in sterile 100µl ddH2O, and kept at -20°c for later use. DNA concentration and purity were measured using Nanodrop spectrophotometer1000 by loading 1µl of samples. Data were statistically analyzed using one-way ANOVA, Minitab 15. All aseptic conditions were maintained while running DNA extraction protocol and to maintain quality.

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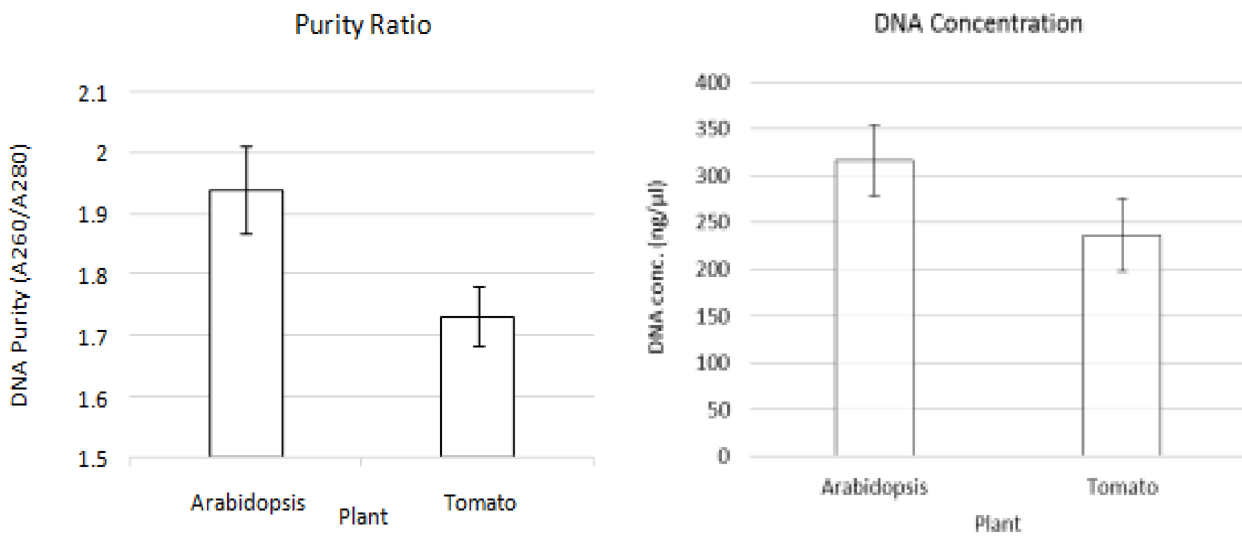
Results and discussion

In an attempt; as a first step, to isolate pure nuclear DNA from tomato valid for PCR analysis and other molecular biology tests, Edwards' buffer was used to extract DNA from tomato comparing to Arabidopsis which has been intensively and successfully tested with this protocol. Clear white jelly DNA pellets were collected from Arabidopsis samples, while DNA pellets from Tomato samples developed visible coloration (dark-brown). Tomato plants are known to contain high level of polyphenol (Peterson, *et al.*, 1997) which is usually responsible for the dark color when destroyed tissues are exposed to oxygen. The development of brown color was prevented when another protocol containing more chemicals was used to block the oxidation. reaction between phenolic compounds and the extracted DNA from tomato samples Comparing to Edwardes' buffer, protocol developed by Peterson et al. seemed to be more expensive as it recruits more chemicals and is a time-consuming procedure. Results show significant difference in DNA purity between the two plant species (P value = 0.000). It was higher in Arabidopsis (1.93±0.072) than in Tomato (1.73±0.048) (Figure 1). Also, there was significant difference (P value= 0.011) in DNA concentration measured as ng/µl) between the two plant species. It was 316.38±38.09 ng/µl for Arabidopsis and 236.71±38.78 ng/µl for tomato (Fig. 1). The high purity of DNA of Arabidopsis achieved by using Edwards' protocol shows that the DNA can be used not only for PCR to determine genotype and gene amplification (Elhaj 2009), but also for Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphisms (RLFP) analyses, GMs and polymorphism detection. The presence of phenolic compounds represents an obstacle against the use of the isolated DNA for molecular analysis. As secondary products Polyphenols interfere with the genomic DNA isolation procedures and prevent further DNA amplification (Amani *et al.*, 2011). Thus, for the collection of clear DNA pellets from Tomato using Edwards' buffer method; it is suggested to add some other chemicals (Barbier *et al.*, 2019) to exclude the phenolic materials from samples as a part of the extraction protocol.

Fig.1: A-DNA purity ratioof Arabidopsis and Tomato leaf samples; B- DNA concentration of Arabidopsis and Tomato leaf samples using Edwards' buffer



A B

Although several rapid DNA isolation protocols are available, they have not been tested for many specific plants. In a comparison to other rapid nucleic acid isolation protocols described for plant samples, Edwards' buffer method represents a simpler and safer procedure. Our try was to describe a method to isolate genomic DNA especially for Tomato plants - which is economically known as an important vegetable crop, to help facilitating molecular investigations in order to understand its genomic clues. Referring to the showed results and having found significant differences comparing to Arabidopsis, it appears that tomato plants needs extra work to exploit the best protocol that help us to collect usable DNA by excluding the natural products that affect the DNA purity and concentration. To keep the simplicity of the use of Edwards' protocol, we anticipate that the utilization of some extra chemicals to the protocol (as a second step) will improve this technique in collecting pure genomic DNA which helps in the genomic studies in such economic plant crops.

References

1. Amani J., Kazemi R., Abbasi A.R. and Salmanian A.H. 2011. A simple and rapid leaf genomic DNA extraction method for polymerase chain reaction analysis. Iranian J. Biotech. 9: 69-71.
2. Barbier F.F., Chabikwa T.G., Ahsan M.U., Cook S.E., Powell R., Tanurdzic M. and Beveridge C.A. 2019. A phenol/chloroform-free method to extract nucleic acids from recalcitrant, woody tropical species for gene expression and sequencing. Plant Methods 15: 62.
3. Berendzen K., Searle I., Ravenscroft D., Koncz C., Batschauer A., Coupland G., Somssich I.E. & Ülker B. 2005. A rapid and versatile combined DNA/RNA extraction protocol and its application to the analysis of a novel DNA marker set polymorphic between Arabidopsis thaliana ecotypes Col-0 and *Landsberg erecta*. Plant Methods. 1: 4.
4. Chabi Sika K., Kefela T., Adoukonou-Sagbadja H., Ahoton L., Saidou A., Baba-Moussa L., Jno Baptiste L., Kotconi S.O. and Gachomo E.W. 2015. A simple and efficient genomic DNA extraction protocol for large scale genetic analyses of plant biological systems. Plant Gene., 1: 43-5.
5. Cheng Y.-J., Guo W.-W., Yi H.-L., Pang X.M. & Deng X. 2003. An efficient protocol for genomic DNA extraction fromCitrus species. Plant Mol. Biol. Report. 21: 177-8.
6. Edwards K., Johnstone C. and Thompson C.1991. A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Res. 19: 1349.
7. Elhaj A. 2009. Molecular and physiological investigations of Arabidopsis mutant lines under abiotic stresses. PhD thesis, unpublished. Newcastle University, Newcastle upon Tyne.
8. Elhaj A. 2021. T-DNA insertion in the KUP8 targeted gene improves the survival under salt stress in the *Arabidopsis thaliana*. J. Genet., Genomics, Plant Breed., 5(1):2-7.
9. Fulton T.M., Chunwongse J. & Tanksley S.D. 1995. Microprep protocol for extraction of DNA from tomato and other herbaceous plants. Plant Mol. Bio. Reporter 13: 207-9.
10. Hu W. and Lagarias J. 2020. A one-tube method for rapid and reliable plant genomic DNA isolation for PCR analysis. doi: https://doi.org/10.1101/2020.02.13.948455
11. Jobes D.V., Hurley D.L. and Thien L.B. 1995. Plant DNA isolation: a method to efficiently remove polyphenolics,

polysaccharides, and RNA. TAXON., 44: 379-86.

12. Kasajima I., Ide Y., Ohkama-Ohtsu N., Hayashi H., Yoneyama T. and Fujiwara T. 2004. A protocol for rapid DNA extraction fromArabidopsis thaliana for PCR analysis. Plant Mol. Biol. Reporter. 22: 49-52.
13. Peterson D. G. Boehm, K.S. and Stack,S.M. 1997. Isolation of milligram quantities of nuclear DNA from tomato (*Lycopersicon esculentum* Mill), a plant containing high levels of polyphenolic compounds. Plant Mol. Biol. Rep. 15: 148-53.
14. Semagn K. 2014. Leaf tissue sampling and DNA extraction protocols. Methods Mol. Biol. 1115: 53-67.
15. Vilanova S., Alonso D., Gramazio P., Plazas M., García-Fortea E., Ferrante P., Schmidt M., Díez M.J., Usadel B., Giuliano G. and Prohens J. 2020. SILEX: a fast and inexpensive high-quality DNA extraction method suitable for multiple sequencing platforms and recalcitrant plant species. Plant Methods. 16: 110.