Simple and Effective DNA Extraction Methods from Thrips

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OPEN ACCESS

Keywords

Thrips, DNA extraction, Hot ShoT method, Lysis Method, DNeasy® Blood and Tissue Kit

How to cite this article:

Manideep, S., Kumar, T. S., Nandini, B. and Akhil, V. S. 2024. Simple and Effective DNA Extraction Methods from Thrips. *Vigyan Varta* 5(12): 150-154.

ABSTRACT

Thrips, as significant agricultural pests, cause extensive crop damage through feeding and the transmission of plant viruses, resulting in considerable economic losses. Efficient DNA extraction is essential for molecular identification and virus detection but is often challenging due to their small size, hard cuticle, and contamination from plant-derived substances. Various DNA extraction approaches have been developed to address these challenges, including alkaline lysis, enzymatic digestion, organic solvent-based methods, and spin-column technology. Alkaline lysis methods provide a rapid and cost-effective solution, yielding DNA suitable for applications like PCR, though additional purification may be required for sensitive analyses. Enzymatic digestion, employing agents like proteinase K, ensures relatively pure DNA that is stable for storage and ready for downstream applications. Organic solvent-based methods, such as CTAB with chloroform phase separation and alcohol precipitation, are highly effective for isolating high-quality DNA, especially in samples with significant contaminants. Spin-column-based commercial kits further simplify the process, delivering DNA with exceptional purity by minimizing impurities, making them ideal for sensitive and high-throughput applications. The choice of a DNA

extraction method depends on the research requirements, balancing time, cost, and the desired quality of DNA. Simpler methods are suitable for routine tasks or large-scale studies, while advanced techniques are preferred for experiments demanding high-purity DNA. Together, these methods offer flexibility and reliability for extracting DNA from challenging specimens like thrips.

INTRODUCTION

Thrips are small, soft-bodied insects that are significant agricultural pests, damaging crops through sap-sucking and serving as vectors for various plant viruses. These insects cause significant economic losses due to their invasive nature and widespread crop damage. For example, the Western flower thrips, *Frankliniella occidentalis*, causes annual losses of approximately US$50 million in the Netherlands, while the Tomato spotted wilt virus (TSWV) transmitted by this species results in global economic damages estimated at US$1 billion (Rugman-Jones *et al*. 2010; Adkins *et al*. 2000). Similarly, other thrips species are known to vector viruses that cause substantial crop yield losses. As such, efficient monitoring and management of thrips populations are critical for mitigating their impact.

Thrips exhibit limited morphological distinctiveness and often have a broad host range, making traditional taxonomic identification challenging and reliant primarily on molecular techniques (Rubinoff *et al*. 2006). Identification keys are generally insufficient for eggs, larvae, and pupae, restricting morphological assessment largely to adult specimens (Mound and Masumoto 2005; Kadirvel *et al*. 2013). Molecular identification offers an effective alternative, being cost-efficient, rapid, suitable for extensive screening, and not dependent on a morphological specialist (Rubinoff *et al*. 2006). Several genes, including 18S rRNA, 28S rRNA, internal transcribed spacers (rDNA ITSs), and mitochondrial cytochrome c oxidase I (mt COI), are now commonly used for species identification in thrips (Asokan *et al*. 2017). Molecular techniques, including DNA extraction, play a key role in the identification and study of thrips, as well as in the detection of the plant viruses they transmit. The DNA extraction process is the first step in many molecular biology assays, such as PCR and sequencing, which are used for species identification, population studies, and virus detection. However, extracting DNA from small, soft-bodied insects like thrips can be challenging due to their tiny size, the presence of a hard cuticle, and contaminants like plant phenolics and tannins that can interfere with DNA recovery and purity. Traditional DNA extraction methods, though effective, are often time-consuming, labour-intensive, and expensive, especially when working with small insect specimens. As a result, there is a growing need for rapid, cost-effective methods to isolate high-quality DNA from thrips that can be used for routine molecular applications.

Methods:

Hot-Shot method:

Aliquot 30 µL of alkaline lysis buffer (25 mM NaOH, 0.2 mM disodium EDTA, pH 12.0) is added to 1.5 mL microcentrifuge tubes containing thrips specimens. Specimens are crushed with a micropestle for 5–10 minutes to release DNA, followed by the addition of 45 µL of lysis buffer to rinse the pestle and ensure complete recovery. The tubes are incubated at 95°C for 15 minutes to lyse cells, then cooled to 4°C for 15 minutes. To neutralize the

solution, 75 µL of neutralizing buffer (40 mM Tris-HCl, pH 5.0) is added. Samples are centrifuged at 11,000 rpm for 5 minutes, and the supernatant containing DNA is collected for PCR and downstream analysis. (Montero-Pau *et al*.,2008)

Lysis Method:

Thrips specimens (5–10 per sample) are placed in 1.5 mL microcentrifuge tubes with an equal volume of lysis buffer (0.2 M NaCl, 0.2 M Tris–HCl, pH 8.0, 1 µL β-mercaptoethanol, 1 µL proteinase K, and 25 µL nuclease-free water). Specimens are thoroughly crushed with a micropestle, and the mixture is briefly spun to ensure even distribution. The samples are incubated at 65°C for 2.5 hours with with gentle tapping every fifteen minutes to ensure even distribution of heat. Following incubation, the temperature is increased to 100°C for 7 minutes to inactivate proteinase K. Extracted DNA is stored at -20°C for further use. (Holterman *et al*., 2006).

CTAB Method:

CTAB extraction buffer is prepared using 10 ml solution of CTAB buffer consisted of 3.5 ml 10% CTAB, 2.8 ml 5 M NaCl, 1 ml 1 M Tris-HCl, 400 µl 0.5 M EDTA (pH 8.0), 20 µl β-mercaptoethanol, and 2.28 ml sterile distilled water (SDW). Single thrips specimens are placed in 1.5 mL tubes, crushed with 20 µL of extraction buffer, and mixed with an additional 80 µL of buffer. Samples are incubated at 65°C for 1 hour with intermittent vortexing. After incubation, an equal volume of chloroform:isoamyl alcohol (24:1) is added, mixed, and centrifuged at 14,000 xg for 15 minutes. The aqueous phase is transferred to fresh tubes, 40ul volume of chilled isopropanol was added before placing the samples at -20°C for 30 minutes or overnight incubation. The DNA was then pelleted by centrifuging at 14,000 xg for 10 minutes, and the supernatant was gently removed. The pellet was washed with 500ul 70% ethanol and decant the ethanol without disturbing the pellet and allowed to air dry, and dissolved in 20 µl Nuclease free water for yield and purity assessment, as well as PCR amplification (Priti *et al*., 2020)

DNeasy® Blood and Tissue Kit

DNA extraction from single adult female specimens using the DNeasy® Blood and Tissue Kit according to the manufacturer's protocol. Place thrips specimens in a 1.5 ml microcentrifuge tube, crush in 180 µl of ATL buffer using a sterile micropestle, and then add 20 µl of Proteinase K. The mixture was incubated at 56°C for 1 hour. After incubation, add 200 µl of AL buffer and 200 µl of molecular-grade ethanol, mixed by vortexing, and transferred to a DNeasy® Mini spin column. The column was centrifuged at 6000 xg for 1 minute, and the flow-through was discarded. Then, 500 µl of buffer AW1 was added, followed by centrifugation at 6000 xg for 1 minute, with the flow-through again discarded. Next, add 500 µl of buffer AW2, and the column was centrifuged at 20,000 xg for 3 minutes. The column was then placed in a fresh 1.5 ml microcentrifuge tube and left at room temperature for 10 minutes. DNA was eluted by adding 20 µl of Buffer AE to the center of the column and centrifuging at 6000 xg for 1 minute. The extracted DNA was then used for yield and purity assessment, as well as for PCR amplification (Jangra and Ghosh, 2022)

CONCLUSION:

DNA extraction methods can vary in suitability depending on purity, efficiency, and application. The **HotSHOT method** offers a rapid approach to extracting DNA, using alkaline lysis buffer and heat incubation to disrupt cells. This method provides DNA suitable for PCR, though its moderate purity may require further cleanup for sensitive

downstream applications. The **lysis buffer method**, involving Proteinase K digestion followed by heat inactivation, yields relatively pure DNA. This method is effective for multiple samples, producing DNA that is stable for storage and directly usable for PCR. The **CTAB method** is good in extracting high-quality DNA with minimal impurities. Phase separation with chloroform:isoamyl alcohol and precipitation with isopropanol ensure DNA with good purity, while ethanol washing further enhances its quality. This method is particularly suited for samples with complex contaminants, enabling effective PCR amplification. Lastly, commercial kits like the **DNeasy® Blood and Tissue Kit** consistently produce high-purity DNA through spin-column technology, minimizing contamination. These kits are reliable for high-quality extractions and are especially effective for single specimens. The choice of method depends on the balance between time, cost, and DNA quality. Rapid and affordable methods like HotSHOT and lysis are ideal for routine applications, while CTAB and commercial kits are preferred for high-purity requirements. Researchers can select the most appropriate approach based on experimental needs and available resources.

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