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## ORIGINAL ARTICLE

# An Efficient and Rapid Method of Genomic DNA extraction from a single Chilli Thrips *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae)

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**ABSTRACT**

*Because of the minute size of thrips, genomic DNA extraction from single specimen is a challenging task. We demonstrated a rapid, simple, efficient and economical procedure to obtain good quality DNA from single adult of chilli thrips. To determine the effectiveness of this method, genomic DNA was checked by nanodrop and agarose gel electrophoresis, which revealed its high quality in sizes above 10 kb without contamination. Nano drop results showed a maximum concentration of 43.2µg / mL (A260/A280 ratio) with a purity range of 1.79 (A260/A230 ratio) from a single specimen. This suggests that the DNA concentration and purity obtained by the new modified method was extremely suitable for molecular biology applications. Apart from this, the present method is rapid, economical, time saving and uses less toxic chemicals with fewer steps. PCR amplification was achieved using mitochondrial COI gene primers and subsequently PCR product was cloned and sequenced.*

***Key words:*** *COI gene; genomic DNA; Scirtothrips dorsalis; modified CTAB; PCR*

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## INTRODUCTION

Many studies require isolation of genomic DNA from single specimen *viz.,* research on systematic, phylogenetics, ecological genetics and molecular methods for detection and control of insect pests [3, 4]. DNA quality is a vital event for most amplification-based analysis, often the DNA amplification is influenced by the presence of co-purifying inhibitors from the matrix or extraction reagents, which can reduce subsequent PCR efficiency. DNA damage may also occur during the extraction procedure due to oxidation and enzymatic hydrolysis, associated with extraction buffers formulation and also excessive mechanical shearing [13]. Currently, most DNA isolation methods are designed primarily for large organisms [8, 10] and seldom are designed for small insects such as Hymenoptera [18]. DNA extraction on very small insects has rarely been described.

Chilli thrips, *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae) is a highly polyphagous plant pest causes damage *via* direct feeding and indirect damage through vectoring tospovirus transmission. Important crop hosts include tea, cotton, mango, citrus, rose, grapes, peanut, and pepper [6, 11]. Till date very scanty information is available on the genetic diversity within *S. dorsalis* populations. Globally most genetic studies of the species have focused on developing molecular identification reactions *via* PCR

and/or restriction fragments [7, 11, 19, and 20] with generally ≥2 individuals per population sequenced at a given locus. In the present investigation an attempt was made to report a modified extraction method for total DNA extraction from single chilli thrips.

## MATERIALS AND METHODS

### Solutions and Buffers

Stock solutions were prepared as follows: 2x CTAB (cetyltrimethylammonium bromide) extraction buffer 100 mM Tris-HC1 (pH 8.0), 1.4 M NaCl, 20 mM EDTA, 2% (w/v) CTAB, 0.2% (v/v) B-mercaptoethanol, Chloroform/Isoamyl alcohol (24:1). TE Buffer contained 10 mM Tris HCL and 1 mM EDTA (pH 7.5).

### Insect collection

*S. dorsalis* were sampled by tapping the young leaves on a white board. The dislodged samples were collected in 95% ethyl alcohol and stored at -20°C until processing. Information about the geographic locations (IIHR, Bengaluru, Dakshina Kannada, Chetthalli) host plant (Chilli and Rose), date of collection, collectors name were marked using pencil. *S. dorsalis* were identified using the morphological keys described in Chandra et al 2010.

### Extraction of Genomic DNA

We modified the methods of CTAB as follows:

Grind the insect to a fine powder using a micro pestle in an eppendorf tube (Preferably 1.5 mL eppendorf tube)

↓

Add 250 µl of CTAB Buffer, Incubate the mixture for 30 min at 65°C, Mix 2–3 times during incubation by inverting tube

↓

Add equal volume of (250µl) of Chloroform: Isoamyl alcohol (24:1), Centrifuge at 13000 rpm for 6 min.

↓

Separate supernatant and add an equal volume of (250µl) of ice cold Iso propanol, centrifuge at 13000 rpm for 10 min.

↓

Dry the pellet and dissolve it in milli Q water/TE buffer (20µl)

### Yield, purity and integrity of total DNA

The quality and quantity of the isolated DNA were determined by nanodrop analysis (Thermo Scientific, USA). The DNA purity was found from the ratios 260/280 nm (an indicator of protein contamination) and 260/230nm (indicator of organic solvent residues). The size and intactness of the isolated DNA was checked by agarose gel electrophoresis by loading the DNA on 1% gel stained with ethidium bromide (1 µg/µl) and run for 30 min at 60 V by using a 1kb DNA ladder (Fermentas). For image acquisition, a gel documentation system was used (Syngene, UK).

### PCR amplification

The mtDNA amplification reactions was carried out using a thermal cycler (Eppendorf, CA) in 25 µl volume containing 3 ul genomic DNA, 1 ul of 10 mM dNTP, 0.7 ul of 10pM primer, 2.5 ul of 10× reaction buffer, 0.7 ul of 25mM Mgcl2, 0.3 ul of DMSO, 1 ul of 2 unit of Taq DNA polymerase (Genie) with the following conditions: initial denaturation at 94°C for 5 min, 35 cycles of (94°C, 40s; 47°C , 45s; 72°C, 60s) and a final extension of 72°C for 10 min. PCR products were visualized after electrophoresis on a 1.5% agarose gel stained with ethidium bromide. The resulting PCR fragment was excised from the gel and purified using the NucleoSpin® ExtractII Kit (MN), following the manufacturer's instruction. MtDNA was assayed through the amplification of a fragment (~750bp) of the cytochrome oxidase I (COI) gene using primer LCO1490:5'-GGTCAACAA ATCATAAAGATATTGG-3' and HCO 2198:5'-TAAACTTCAGGG TGACCA AAAAATCA-3.

### Cloning, and sequence analysis of the mtCOI gene

The standard recombinant DNA techniques used in cloning were according to Sambrook and Russell[15]. The *E. coli* DH5α cells were transformed with PCR-amplified ~750bp mtCOI gene ligated in pTZ57R (T/A cloning vector) using Fermentas DNA ligation kit (#K1214). The transformed cells (20µl) were spread on LB agar plates containing X-gal (270 µg/ml), IPTG (120 µg/ml) and ampicillin (100 µg/ml). The plates

were then incubated at 37$^0$C for 24 h to screen blue and white colonies. Cloning was confirmed by colony PCR, plasmid mobility check and restriction analysis of recombinant plasmid DNA containing mtCOI gene. The mtCOI gene was sequenced, to determine the homology with the known sequences in the NCBI GenBank database.

## RESULTS AND DISCUSSION

Reliability, feasibility and reproducibility of molecular genetics studies are often limited by the preliminary step - DNA isolation. The obtainment of good amount and high quality DNA from small quantities of tissue has been often a laborious task. Extraction methods should ideally be straightforward, quick, efficient, and reproducible while minimizing the potential for cross-contamination. It should also be suitable for extracting multiple samples and generate minimal risk for the operator. Further, safety, time and costs are also main criteria to be considered. DNA extraction is a routine step in many insect molecular studies. A variety of methods have been used to isolate DNA from insects and many commercial kits are available. Extraction methods need to be evaluated for their efficiency, cost and side effects such as DNA degradation during extraction.

**Table 1.** Comparative table for features of different genomic DNA extraction methods

| Particulars | Conventional CTAB DNA extraction | Commercial DNA extraction kit | modified CTAB DNA extraction |
|---|---|---|---|
| **Steps (No's)** | 10 | 15 | 5 |
| **Duration of DNA extraction (Hours)** | 4 | 2 | .5 to 1 |
| **Quality (260/230nm)** | 1.68 | 1.70-1.80 | 1.79 |
| **Quality in agarose gel** | Good | Good | Good |
| **Yield (µg/ml)** | | | |
| **Toxic chemical** | Phenol, Isoamyl alcohol and isopropanol,CTAB, Proteinase K | | No involvement of such a toxic chemical like conventional method |
| **Color of DNA pellet** | clear to white | clear to white | clear to white |
| **Economy** | Moderate costly | Costly | less cost |

**Table 2.** Analysis of concentration, wavelength absorbance readings and rates for the biological samples used in the efficiency test

| Sample[1] | Wavelength rates[2] | Wavelength absorbance (A)[3] | | | DNA concentration (ηg µl$^{-1}$) |
|---|---|---|---|---|---|
| | 260/230ηm | 260/280ηm | 230ηm | 260ηm | 280ηm |
| *S. dorsalis* IIHR, Castor (n=1) | 1.79 1.80 | 0.209 | 0.130 | 0.086 | 43.6 |
| *S. dorsalis* IIHR, Grapes (n=1) | 1.71 1.79 | 0.201 | 0.129 | 0.084 | 41.0 |
| *S. dorsalis* Dakshina kannada, Rose (n=1) | 1.70 1.82 | 0.210 | 0.132 | 0.082 | 42.9 |
| *S. dorsalis*, Chettahalli, chilli (n=1) | 1.73 1.70 | 0.200 | 0.128 | 0.074 | 32.9 |

*1 Numbers in brackets indicate the quantity of specimens used.*
*2 Wavelength rate higher than 1.7 indicates pure samples and lower than 1.7 indicate samples with significant levels of buffer/organic components (260/230ηm) and proteins (260/230ηm).*
*3 Absorbance were measured by the optical density of the sample in relation to ultrapure distilled water.*

Genomic DNA from a single chilli thrips was successfully extracted, generally yielded good amounts of DNA and confirmed clearly using the nanodrop and Agarose gel electrophoresis. The efficacy of the method was indicated by amplifying and sequencing a known amplicon. The amplified PCR products showed identical band patterns and similar intensity to each other. The amplifications from specimens which were preserved in 95% ethanol for a shorter time were clearer (Fig. 1). The amplified PCR products from the COI of all specimens were successfully sequenced (Fig. 2) and found to be in expected sizes. Single and stable band for all amplifications were visualized, indicating that DNA extraction with

this protocol is reliable and sufficient for the PCR amplification procedure. According to present investigation results, this method could provide high-quality DNA suitable for research in systematics and evolution. The most important step for the procedure is cell disruption; for the insects are small and to prevent loss and cross-contamination, we ground the specimens inside the tube. The extraction requires less than an hour and can provide sufficient amounts of high-quality DNA for PCR with low temperature centrifugation circumvents the need for liquid nitrogen.

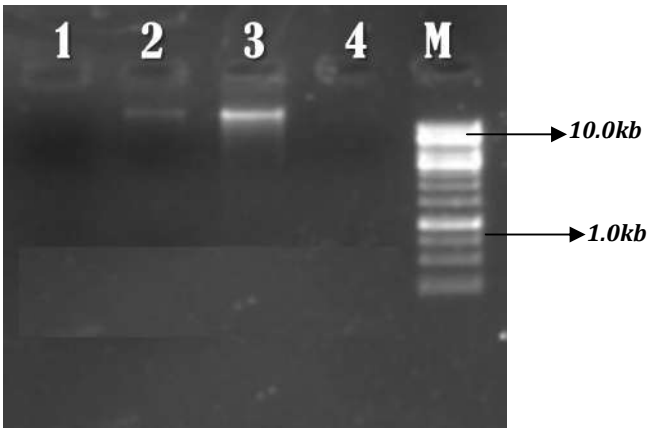


**Fig.1.** The 1% ethidium bromide-stained agarose gel showing DNA samples after DNA extraction from single chilli thrips. Legends: **1**-Intentionally left blank; **2**-*S.dorsalis*, Chettihalli DNA, **3**- *S.dorsalis*, IIHR, Bengaluru, castor DNA; **4**-Intentionally left blank **M**- Molecular Weight Marker (Thermo Fisher Scientific, UAS) 1.0kb DNA ladder

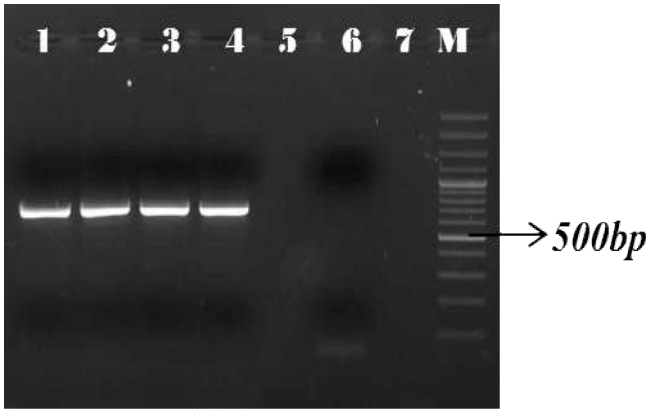


**Fig. 2. Agarose gel electrophoresis (1.5%) PCR amplifications of DNA extraction from 4 specimens of *Scrithothrips dorsalis* using *MtCOI*gene**. Legends:**1-***S.dorsalis* IIHR, Bengaluru, Castor; **2**- S.*dorsalis* IIHR, Bengaluru, Grapes; **3-** *S.dorsalis*, Dakshina Kannada, Rose; **4-** *S .dorsalis*, Chetthalli, Chilli; **5**- Intentionally left blank; **6**- Genomic negative control; **7**- Intentionally left blank and **M**- Molecular Weight Marker (Thermo Fisher Scientific, USA)-100 bp plus DNA ladder.

```
                  10        20        30        40        50        60        70        80        90       100
                  ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
KJ579252_CRIDA-ENT-4/2013
GGCATTTTATATTGTATATTTGGGTTTTGATCTGGTATACTTGGATTATCACTTAGAATATTAATTCGACTTAATCTGCGTGTACCTATAAAAATTTTTA
Bangalore_IIHR_Ricinus cuminu  ....................................................................................
Bangalore_IIHR_Grapes          ..........G.........................................................................
Dakshina Kannada_Rose          ....................................................................................
Chitahalli_chilli              ....................................................................................

                  110       120       130       140       150       160       170       180       190       200
                  ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
KJ579252_CRIDA-ENT-4/2013
TTAGAAATGATCAATTTTATAACTATGTTGTTACAGCTCACGCTTTCATTATAATTTTTTTTACAGTAATACCTATTATAATTGGAGGGTTTGGAAATTG
Bangalore_IIHR_Ricinus cuminu  ..................C.................................................................
Bangalore_IIHR_Grapes          ....G.............C.................................................................
Dakshina Kannada_Rose          ..................C....C............................................................
Chitahalli_chilli              ..................C....C............................................................
```

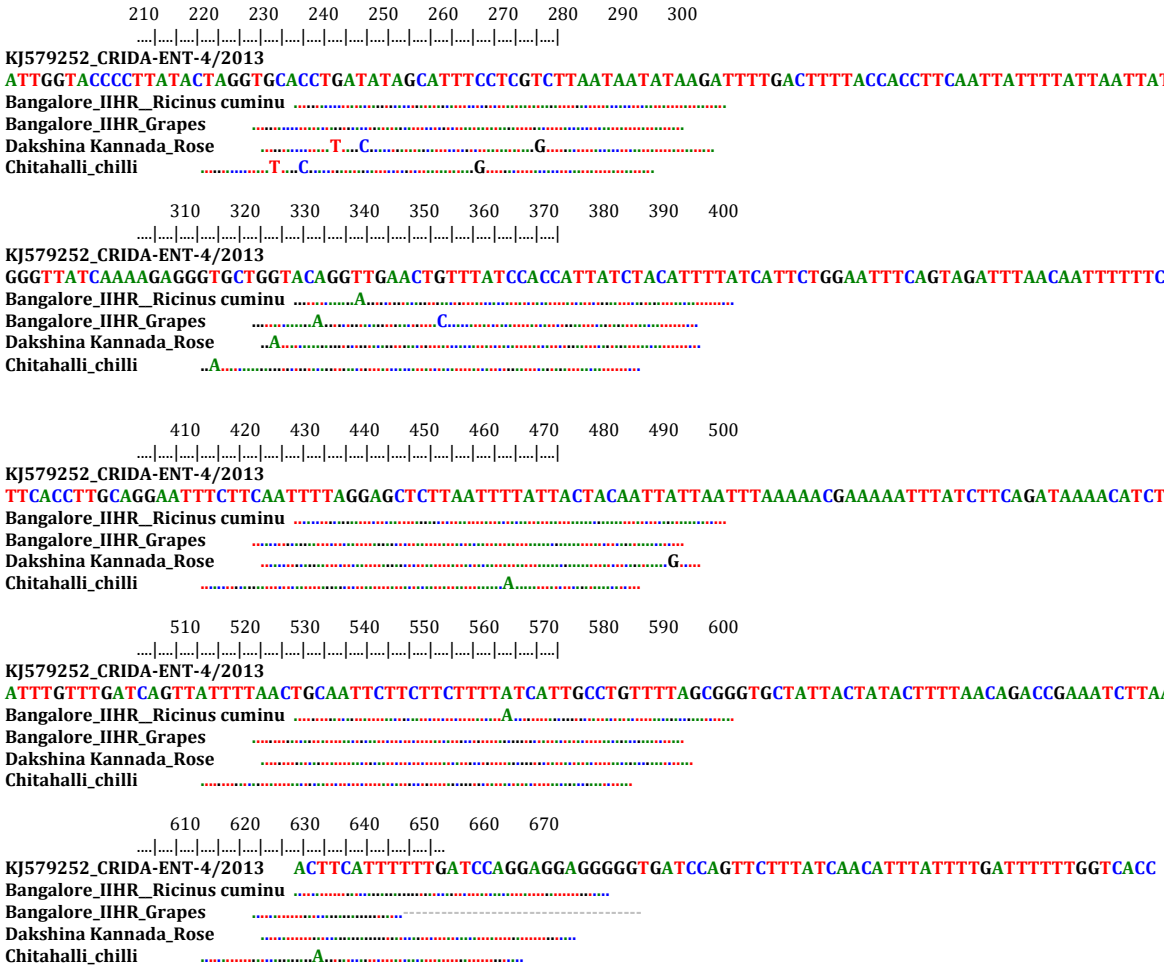


**Fig. 3.** Partial nucleotide COI sequences of Chilli thrips samples. Identities with the NCBI database sequence are indicated by dashes.

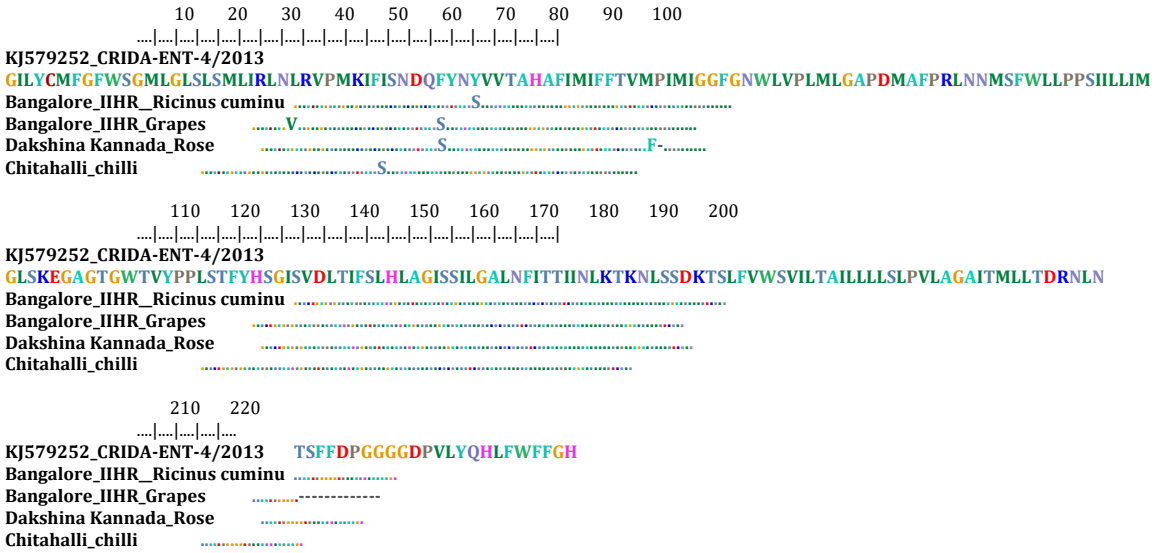


**Fig. 4.** Comparison of the amino acid sequences of mtCOI gene produced with the samples of chilli thrips *S.dorsalis* (GenBank accession number KJ579252). A "." is placed at those positions in new mtCOI gene where the amino acid composition is the same as that in KJ579252 represented in blocks. "-"represents the gaps.

The genomic DNA obtained with the presented methodology was of high quality regarding all standards employed. However, and as described by different authors, the quality and total DNA contents provided

by Nano Drop do not accurately represent the quantity of DNA that is efficiently amplifiable by PCR [9, 16]. In order to analyze the quality of amplifiable DNA, PCR products were cloned into the TA cloning vector pTZ57R/T [15]. Cloning was confirmed through colony PCR and plasmid mobility analysis (Supplementary data), and the clones were sequenced. The sequences obtained were aligned and compared with *S. Dorsalis* mtCOI gene sequences in NCBI GenBank with the accession number KT324659 (Bengaluru_IIHR_Ricinus Communis), KT324660 (Bengaluru_IIHR_Grapes), KT324661(Dakshina Kannada_Rose) and KT324662 (*Chetahalli_Chilli*), revealed 96–99 % homology with previously identified *S. dorsalis* mtCOI genes.

DNA yield is influenced by many factors such as species, type of tissue, method of preservation, extraction procedure, and precipitation method. Tian and Yu [18] reported that for very small insects, shorter storage time in 95% ethanol is an ideal parameter. Although Proteinase K and Chelex 100 are rapid and easy to perform Wang and Wang [21] reported that CTAB method provides higher purity of DNA. The quality and quantity of extracting DNA could be affected by the incubation temperature for lysate. Shahjahan et al. [17] found that the incubation at 37˚C resulted in more than double the amount of total DNA when compared to the incubations at higher temperatures. However, the effect of losses on 37˚C incubation needs to be verified because the higher temperature ranging from 55 to 65˚C is commonly used in the SDS and CTAB methods [2, 5, 9, 12, 14]. As an alternative to ethanol, one volume of 100% ice cold isopropanol is often used to precipitate DNA because the precipitation efficiency of this chemical is higher than that of ethanol [1, 15].

## CONCLUSION

Different methods of genomic DNA isolation effects on quality of the DNA template for further use [5]. An ideal extraction technique should optimized for good DNA yield, minimize degradation, and be efficient in terms of cost, time, labour, and supplies. It should also be suitable for extracting multiple samples and generate minimal hazardous waste. The current novel technique is simple and quick, utilizes fewer specimens for identification, provides high yield of DNA and can be easily mastered by non-experts. In conclusion, the overall results of our method provide a robust podium for the isolation of high quality genomic DNA from a single insect. The time required for the extraction is ~50-60 minutes and the procedure also has been validated by inter-laboratory researchers. The purpose of this method is to establish an economical, simple and rapid method for extracting genomic DNA from a single chilli thrips. In future this modified simple and rapid DNA extraction procedure could be applied to studies of many other small insects across a wide range of orders with suitable modifications.

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