EVALUATION OF DIFFERENT METHODS OF NUCLEAR DNA EXTRACTION FOR *LYGUS HESPERUS* SAMPLES PRESERVED IN VARIOUS STORAGE CONDITIONS

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Abstract

The quality and quantity of Dioxyribonucleic acid (DNA) samples of *Lygus hesperus* are very important for various molecular biology studies. Two common nuclear DNA extraction methods (Phenol chloroform and Cetyltrimethylammonium Bromide (CTAB) method) and one commercial kit (MasterPure DNA purification Kit from Epicenter Biotechnologies) were evaluated for *Lygus* specimens preserved in two different storage conditions (frozen and fresh). Ten adult *Lygus* samples were used for each method. DNA samples were quantified and quality was assessed by photosphectometry and agarose gel electrophoresis. MasterPure kit yielded higher quantity of DNA compared with Phenol-Chloroform and CTAB method. Higher amount of DNA was obtained from fresh *Lygus* samples as compared with frozen samples when CTAB method was used.

Introduction

Molecular biology can answer many questions which can not be answered by general biology or ecological studies. Therefore molecular approaches have become an indispensable tool for entomologist studying insect systematics, molecular evolution, population genetics or plant-insect interactions. Molecular biology includes studies on variation in DNA, Ribonucleic acid (RNA) or Proteins. High quality DNA is required for the molecular systematics of any organism (Salah et al.1997). Isolation of the total genomic DNA is the first step in molecular experiments. The different methods of DNA extractions are being used in different organisms for different tissue types. At one hand, a good DNA extraction procedure should be as simple, safe and inexpensive as possible and on the other hand it should furnish DNA of sufficient quality and quantity for subsequent analysis. So far no appropriate DNA extraction protocols for *Lygus hesperus*, an important pest of cotton in the Texas high plain has been reported. Therefore in this study we have evaluated three different DNA extraction protocols on *Lygus hesperus* with two tissue types, fresh *Lygus s*ample and frozen *Lygus* sample.

Materials and Methods

The DNA sample was extracted from the whole body of a single adult *Lygus* bug. Ten samples of frozen (preserved at -20 ^{o}C) and fresh insect from the laboratory reared Colony were used for DNA extraction using three different methods; Phenol-Chloroform, CTAB and Master Pure DNA kit. DNA quantity and purity was assessed using Biophotometer and quality of DNA was assessed with agarose gel electrophoresis. Electrophoresis was done using 1% agarose and 0.5X Tris-buffered Saline and was run at 100 volts for 1 hr.

A. Phenol –Chloroform (Reineke et al. 1998)

Whole adult *Lygus* was ground in 500 µl grinding buffer (50 mM Tris pH 7.0,0.1M NaCl, 0.1M EDTA, 5%SDS) and 5 µl Proteinase K (25 µg/µl) was added. Then the samples were incubated for 3hr at 37 °C. It was extracted once with phenol, centrifuged for 5 minutes at 3500 rpm the supernatant was transferred to fresh tube and extracted with Chloroform: Isoamyl lcohol (24:1) and centrifuged at 3500 rpm for 5 minutes. Then the supernatant was transferred to fresh tube and 1/10 volume of 3 M NaAc was added and was precipitated with 2 volume of ethanol overnight at -20 °C. Samples were centrifuged for 10 minutes, at 4 °C at 14000 rpm. The DNA pallet was washed with 70% ethanol, air dried and finally re-suspended in 50 µl TE buffer.

B. CTAB (Reineke etal., 1998)

Whole *Lygus* was grind in 500 µl of buffer containing 0.1M Tris, pH 8.0,10 mM EDTA, 2% SDS; then 5 µl of 25µg/µl Proteinase K was added. The samples were incubated at 58 °C for 1 hr. Then 140 µl of 5 M NaCl and 65µl of 10% CTAB were added and the samples were again incubated for 10 min at 65 °C. The samples were extracted with Chloroform: Isoamayalalcohol (24:1). The supernatant was transferred to fresh tube and 280 µl of 5M Ammonium acetate was added. The samples were incubated for 30 min on ice, centrifuged for 20 minutes at 14000 rpm and then precipitated with 500 µl of 30% PEG (Polyethylene glycole). Samples were centrifuged for 10 minute, 4 °C at 14000 rpm. Then pallet was washed with 70% ethanol, air dried and finally resuspended in 50 µl TE buffer.

C. Master Pure DNA extraction kit

The protocol provided by the manufacturer was followed. Whole *Lygus* was grinded in 300 µl Tissue and Cell Lysis Solution and 5 µl of Proteinase K from 25 µg/µl stocks was added. Samples were incubated at 65 °C for 15 min and 175 µl of MPC protein precipitation Reagent was added and samples were precipitated with 500 µl of 100% Isopropanol.

Results

There was no significant difference in quantity of DNA extracted from fresh samples from all three different methods (Phenol Chloroform, CTAB and MasterPure method). Where as in frozen samples; MasterPure yielded highest average quantity of DNA (4 µg/insect) followed by Phenol Chloroform (3.2 µg/insect) and CTAB (2.3 µg/insect) of DNA (Figure 1).

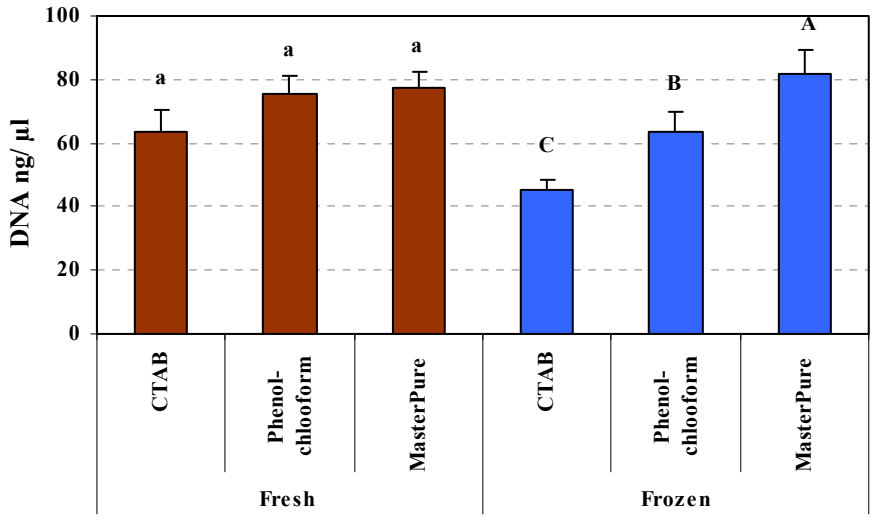

Figure 1. Quantity of *Lygus hesperus* DNA extracted by different methods

CTAB method yielded significantly more DNA from fresh samples than from frozen samples. Similar amount of DNA was yielded from fresh and frozen samples when the DNA was extracted by both Phenol Chloroform and MasterPure DNA extraction kit method (Figure 2).

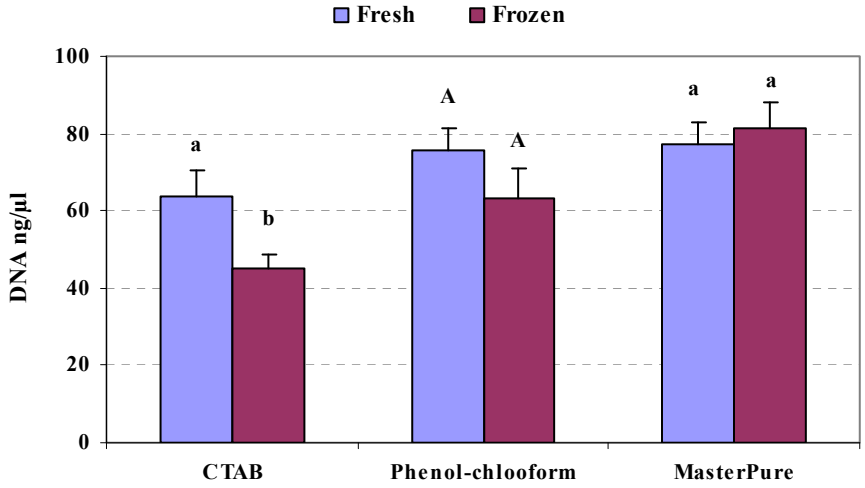

Figure 2. The quantity of DNA extracted from fresh and frozen *Lygus hesperus* saples

The purity of DNA was determined from the A260/A280 ratio. The DNA samples which were most pure should have the 2.0 A260/A280 ratio. In an average all DNA extraction methods yielded closer to pure DNA (1.8 A260/A280 ratio) from frozen *Lygus* samples where as the ratio was lower (1.5 A260/A280 ratio) incase of DNA was extracted from fresh samples. It indicated that DNA samples obtained from fresh tissue had more protein contamination than that from frozen sample. In fresh Sample CTAB gave significantly more pure DNA (averaged 1.9 A260/A280 ratio) than other two methods (Figure 3).

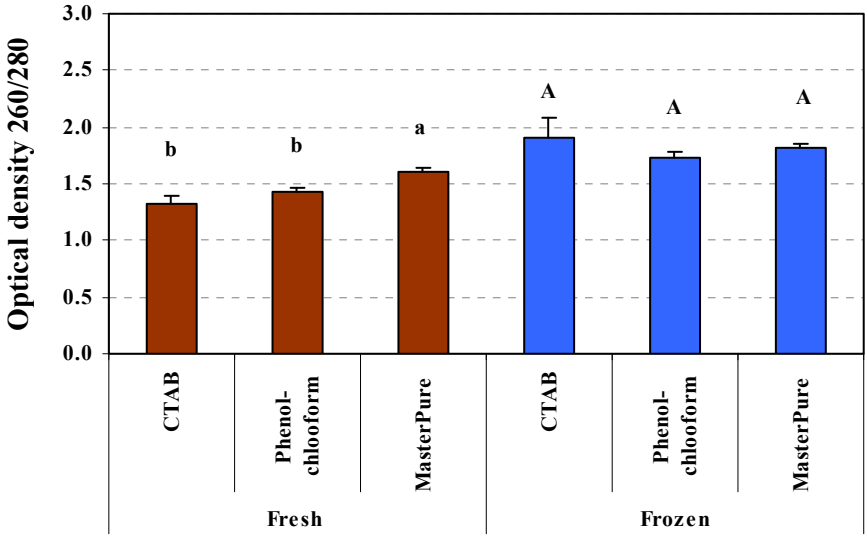

Figure 3: The photosphectrometric measurments of purity of DNA extracted by different methods from fresh and frozen *Lygus hesperus* samples

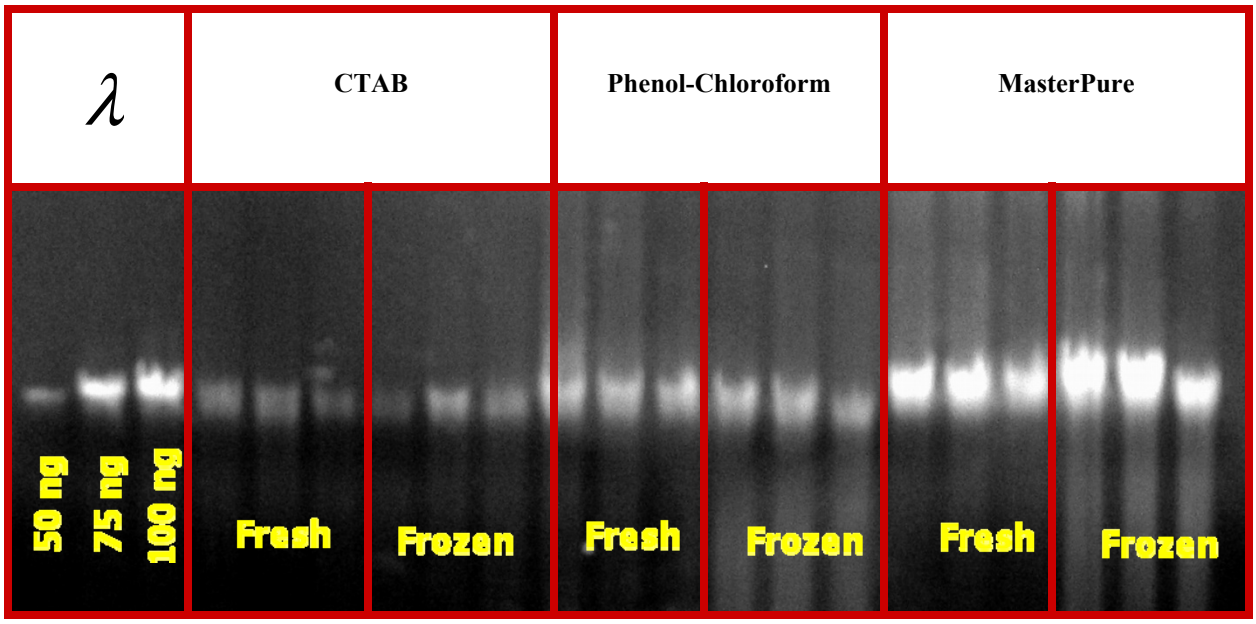

Figure 4. The DNA quantity and quality assessment by Agarose gel electrophoresis

The quantity and quality of DNA extracted was compared with lambda DNA standards. The quantity of DNA extracted by CTAB method in fresh sample was close to 75ng/ µl and around 50ng/ µl l in frozen sample, by phenol chloroform method quantity of DNA extracted was above 75ng/µl in fresh and frozen and from MasterPure quantity of DNA extracted is above 100ng/ µl (Figure 4).

Summary

MasterPure extraction method yielded higher quantity of DNA compared to Phenol-Chloroform and CTAB methods. Fresh *Lygus* samples gave higher amount of DNA compared to frozen samples when CTAB method was used. Frozen samples gave more pure DNA as compared to fresh samples.

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