

ANALYSIS OF FOUR DIFFERENT METHODS OF DNA EXTRACTION FROM THE TRUE WEEVIL (COLEOPTERA: CURCULIONIDAE)DIVYA SHARMA*, DALIP KUMAR AND RHITOBAN RAY CHOUDHURY¹

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Received : 10.02.2018 ; **Accepted** : 08.04.2018**ABSTRACT**

PCR-based markers have been widely used for the analysis of genetic diversity and to avoid ambiguity, molecular characterization is very effective tool for accurate discrimination and identification of a species in insects. Because these studies require analysis of large number of samples, a DNA extraction method that is fast, inexpensive and yields high quality DNA from the preserved samples, needs to be evaluated. A comparative analysis of four methods for DNA extraction from a single specimen of rice weevil, *Sitophilus oryzae* preserved in 90% alcohol has been communicated. Significantly higher DNA yields were obtained by using SDS-Potassium acetate method followed by CTAB, DNA Xpress and Bioline Isolate II genomic DNA kit. Maximum purity (A260/A280- 1.8) was obtained with Bioline Isolate II genomic DNA kit method. The Absorbance ratio was appreciably low with DNA Xpress kit showing the presence of proteins. Bioline Isolate II genomic DNA kit was time efficient and yielded good quality DNA but at a high cost. Based on DNA yield and quality, these evaluations provide a guide for choosing Bioline Isolate II genomic DNA kit method of DNA extraction for rice weevils and optimizing the extraction conditions for rice weevils.

Figure : 00

References : 08

Table : 01

KEY WORDS : DNA extraction, Genome, Molecular Characterization, *Sitophilus oryzae*.**Introduction**

The DNA extraction is the most important step for the molecular investigations. Molecular analyses have proved to be pivotal in studies related to genetic diversity, evolution, accurate identification and discrimination among the closely allied species. Many methods have been established for extracting DNA from the biological material like tissue, whole insect etc.

Various DNA extraction methods give different results for DNA yield and quality. DNA extraction method should be referred as ideal if it results in optimum DNA yield, cost and time efficiency, less or no hazardous. DNA extraction methods result in different yield and quality of DNA. The optimum method can be evaluated by comparing the results of the different DNA extraction methods¹. The most prevalent traditional methods for extraction of DNA include Sodium Dodecyl Sulfate (SDS) and CetylTrimethylAmmonium Bromide (CTAB) methods. These two methods are time consuming methods. They involve phenol and chloroform which are hazardous

chemicals. DNA Xpress kit involves single buffer that solubilizes all cell components and allows DNA to precipitate in presence of absolute alcohol^{2,3}. Bioline Isolate II Genomic DNA kit has two lysis buffers which lyse the cell components, a washing buffer which stabilises DNA and an elution that elutes the DNA bound with the silica column.

The first step of cell lysis requires heat incubation that ranges from 55p C to 65p C with varying time periods i.e. 30 minutes to overnight. The DNA is precipitated using sodium ions, ethanol or isopropanol from its aqueous solution. Every method uses different volumes and concentrations of ethanol or isopropanol. The temperature for incubation with the precipitants may vary from "20° to 25°C and time may vary from 0 – 15 hours^{2,4,7}. The earlier studies have shown that longer incubation time and lower temperature do not enhance the precipitation of DNA^{5,8}.

In this study, we evaluated the yield and quality of DNA using the four methods. The cost and time required

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for processing were also compared for each sample among by these methods. Our goal is to evaluate these DNA extraction techniques for handling of a large number of small insects such as *Sitophilus oryzae*.

Materials and Methods

Sample collection and preparation weevils were collected from the stored grains from different sites like warehouse, animal feed store *etc.* the samples were preserved in absolute alcohol at 20°C.

DNA extraction protocols

For each method, total DNA was individually extracted from 10 samples (weevils). The colour of the DNA pellet in each tube was recorded. The DNA from single weevils was re-suspended in 100 ml of TE buffer of pH 8 except for the DNA obtained using the Isolate II Genomic DNA kit which suspend extracted DNA in elution buffer. The extracted DNA samples were stored at 20°C for further analysis. In SDS method weevil was grounded in grinding buffer that consisted of 0.1M NaCl, 0.2 M Sucrose, 0.1M Tris-HCl, 0.05M EDTA and 0.5% SDS [10]. After incubation of 30 minutes at 65°C 8M Potassium Acetate was added. These contents were incubated for 30 minutes and centrifuged for 15 minutes at 10,000 rpm. Supernatant was separated and double volume of chilled ethanol was added. The sample was left overnight at 20°C. The next day sample was centrifuged to get DNA pellet which was washed in ethanol and dissolved in 18 ml TE buffer and stored at 20°C.

In CTAB method the lysis buffer has 2% diluted CTAB in 100mM Tris-HCl, 20mM EDTA and 1.4M NaCl with 0.2% β -mercaptoethanol. After grinding the weevil in CTAB lysis buffer incubate it for 30 minutes at 55°C in water bath. Then chloroform was added and after centrifugation the top layer was transferred to another tube. 7.5M Ammonium acetate and Isopropanol was added and incubated on ice for 30-40 minutes. DNA pellet thus formed was washed by 75% and 95% ethanol. DNA pellet was dissolved and stored in TE buffer (pH-8) at 20°C.

DNA Xpress method was performed as provided in the user manual by HIMIDEA. The weevil was kept in SDS digestion buffer supplemented with Protienase K and incubated for 4 hours at 55°C. After centrifugation the supernatant was transferred to new tube and then 0.5ml of DNA Xpress reagent was added and incubated at 25°C for 5 minutes. 1ml of Ethanol (96-100%) was added and after mixing it was allowed to stand for 3 minutes. The eppendorf tube was centrifuged at 85,000rpm for 5 minutes to get DNA pellet. The pellet was washed twice in 95% Ethanol. The pellet was dried using Kim wipe and dissolved in freshly prepared 8mM NaOH. Extracted DNA was stored at 20°C.

Isolate II Bioline kit was also performed as per user manual of Bioline. The weevil was crushed in lysis buffer and kept at 55°C for overnight. The next day sample was transferred to spin column tube. After adding the GW buffer to the spin column were centrifuged. The supernatant was transferred to new micro centrifuge tube. Centrifugation was done after adding the next buffer to get the DNA pellet. The pellet was washed in ethanol and then stored in TE buffer (pH-8) at 20° C.

Estimation of DNA quantity and quality

DNA concentration and quality was measured by Nanodrop spectrophotometer by analysing concentration and the absorbance ratio (A260/A280) respectively. Only 1 μ l sample of DNA was used for concentration measurement. Proteins occur as the contaminants when DNA is extracted from biological samples as proteins are tightly bound to DNA. The peak of UV absorption was at 260 nm for DNA and at 280 nm for protein when analysed by spectrophotometry. A pure sample of DNA has the absorbance ratio for 260 nm and 280 nm is at 1.8 and is relatively free from protein contamination. To compare the efficiency of the DNA extraction methods, the DNA yield from single weevil was calculated based on the DNA concentration and final volume.

Estimation of DNA viability for PCR

28S gene was amplified from each DNA samples basically to assess the DNA quality for PCR application.

Estimation of cost and time

The cost was calculated by evaluating price of chemicals or kits used. The cost does not include the price of disposable items like eppendorf tubes, pipette tubes and also excludes the price of the instruments used like pipette, incubator, thermocycler, geldoc *etc.*

The time was estimated based on different protocols and also included the time for lysis incubation and DNA pellet drying time wherever required. The time spent for solution preparation in the SDS and CTAB methods and autoclaving of the glassware and pipette tips was excluded.

Results

DNA quality and quantity

UV absorbance ratios for the four methods of DNA extraction are compared (Table-1). The yield rates by the SDS and CTAB methods were significantly higher than those obtained by the DNA xpress and Isolate II Genomic DNA kit. The mean ratio of SDS method was nearest to 1.8 while the DNA Xpress ratio mean was the highest (*i.e.* 2 or >2). These results were then analysed as the protein contamination being highest in DNA Xpress method and lowest for SDS method.

DNA pellet colouration

TABLE- 1 : Comparison of four methods of DNA extraction for *Sitophilus*

	SDS method	CTAB method	DNA-Xpress method	Isolate II Bioline Kit
DNA yield(ng/μl)	238 ± 98	116 ± 106	47 ± 30	71.4 ± 7
Absorbance ratio (260/280)	1.88	2 or >2	1.75 or >1	1.15 or >1
Colour of DNA pellet	Light to dark brown	Clear to white	Light brown to clear	Clear to white
Incubation for lysis	30 minutes at 65°C	30 minutes at 55°C	4 hours at 55°C	Overnight at 55°C
Estimated cost per 50 samples	15	30	1020	12,000
Time(excluding overnight incubation)	2hrs 30 min	2hrs 45 min	1 hour	1hrs 30min

The DNA pellet formed after vaporisation of the alcohol as the ultimate product of the DNA extraction. This pellet was preserved by adding dilute buffer like freshly prepared TE or NaOH. The pellet colour was analysed for each protocol. The DNA pellet showed variation in colour (Table-1). The colour of pellet was clear or white in CTAB and Isolate II Genomic DNA kit, light brown in DNA Xpress protocol and light to dark brown in SDS protocol. The colour of the DNA pellet was not related to the degree of any contamination in the extracted DNA sample.

DNA extraction Cost and Time per sample

The estimated cost in rupees¹ and time in hours for each method to extract DNA from a single beetle per 50 samples are listed (Table-1). Due to the much lower expenses of the laboratory prepared SDS and CTAB buffers these two methods were less costly than the two commercial kits. The Isolate II Genomic DNA kit was the most expensive among the four methods but required the least extraction time.

Discussion

The DNA quality and quantity was different within each extraction method and across the four methods. Comparing the extracted DNA product for all the protocols used in this investigation, it has been acceptable in the

reference to the quality and quantity. The extracted DNA sample had higher yield rate for the SDS and CTAB methods as compared to the other two methods. The extracted DNA sample had relatively higher protein concentrations using DNA Xpress and CTAB methods while lowest protein contamination was observed in the Isolate II Genomic DNA kit method. The time duration for the Isolate II Genomic DNA kit DNA extraction of single sample of true weevil was least⁸. So, Isolate II Genomic DNA kit DNA extraction method was analysed to be most time efficient. The CTAB and SDS methods were lengthy while doing the DNA extraction as well as these two involved the preparation of different buffers in the laboratory and as such CTAB and SDS methods were not at all time efficient.

The colour of extracted DNA pellet varied from light brown to dark brown and clear to white. The DNA quality as estimated by Nanodrop did not show any relation to the colour of the pellet.

The DNA sample extracted by the four different methods was amplified for 28S gene. All the extracted DNA samples showed amplification showing the viability of DNA for molecular studies. The cost estimation of DNA extraction for 50 samples by four different methods showed the costliness of Isolate II Genomic DNA kit and the cost effectiveness of SDS and CTAB methods.

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