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OPTIMIZATION OF EXTRACTION DNA FROM CEREAL APHID

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ABSTRACT
In this study, we report a modified SDS method for the extraction of high quality genomic DNA from a single aphid. This method efficiently isolates DNA from small specimens, which is difficult and challenging because of the small amount of starting tissue. Grinding with liquid nitrogen and phenol treatment are eliminated using this methodology. The A260/A280 absorbance ratios of the isolated DNA ranged from 1.67 to 1.94, suggesting that the DNA is pure and can be used for further molecular analysis. This method represents a fast, cheap, and effective alternative method for laboratories with low budgets.

KEYWORDS: *Cereal aphids, genomic DNA extraction, SDS.*

INTRODUCTION

Cereal aphids are a serious problem in wheat growing [1]. A considerable annual variation in aphid population dinamics has been frequently observed. The aphids form a multispecies complex on the cereal crops. Such parallel occurrence of multiple aphid species is determined by similar life history: the species are holocyclic though overwintering on different primary hosts [2]. The aphids migrate to wheat plants around mid-May from their winter hosts Rosa spp. (M. dirhodum), different Poaceae (S. avenae) and Prunuspadus L. (R. padi). Aphids can cause significant yield reduction by means of direct feeding damage and diseases introduced by virus transmission.

Cereal aphids have small size of 3-5mm. Identification of cereal aphids is traditionally based on morphological features, and although several keys exist, it is difficult to distinguish aphids morphologically because of presence of intraspecific variation and similarities between species [3]. It has also been reported that environmental conditions and different host plants species can cause morphological changes in aphids [2]. Furthermore, aphid eggs cannot be identified without culturing in the laboratory and then analyzing key features of the viviparous parthenogenetic female aphids and subsequent viviparous offspring. This process is time-consuming and laborious and aphid eggs have poor hatching rate in the lab. Therefore, application of molecular genetic approaches is necessary for identification of economically relevant aphid species in the cereal fields.

Isolation of genomic DNA is very important issue in the field of molecular biology. Extraction of high-quantity and -quality DNA from small amounts of insect tissue is often a difficult task [4]. The most commonly used DNA isolation methods employ toxic chemicals (phenol, chloroform, β-mercaptoethanol). For example, β- mercaptoethanol, which is being used in some protocols for example in CTAB protocol causes severe eye irritation, is harmful for skin, respiratory tract and central nervous system.

Several protocols for isolation of insects DNA have been published [5-8]. Commercially available DNA isolation kits (DN easy Qiagen, E.Z.N.A.® Insect

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DNA Kit Omega bio-tek) provide higher throughput and reduced labor time, but their availability and high cost can be limiting factor for molecular genetics studies in the Uzbekistan, especially when field surveys of aphid populations require processing of a large number of samples and considering experiments with limited financial resources.

The main objective of this study was to establish an affordable, reproducible and efficient method for isolation of pure,high-yield DNA from aphids.

MATERIALS AND METHODS

Insect material. The aphids were collected from experimental wheat field in the Institute Genetics and Plant Experimental Biology, where a large diversity of aphid population is recorded. A total number of 8 aphid individuals were harvested from wheat leaves and spikes (figure 1).The research was performed in the Cereal Genetics Laboratory of the above mention Institute.

Fig. 1. Cereal aphids from experimental field

Reagents and solutions. Extraction buffer (fresh preparation): 0.5 % SDS; 50 мMNaCl; 100 мМ EDTA; Chloroform : isoamil solution (24:1); Isopropanol; 70% ethanol; 5 M NaCl; TE buffer;

DNA extraction. DNA extraction method was adapted from [9] with modifications. For DNA isolation, entire fresh aphid (2 mg) was transferred to a 1.5 mL eppendorf tube, ground using a plastic pestle, and immediately mixed with 600µl SDS extraction buffer (0.5 % SDS; 50 мМ NaCl; 100 мМ EDTA) and incubated for 60 min at 550C with gentle mixing every 10 min. The samples were left at the room temperature (RT) and add then equal volume of 24:1 chloroform: isoamil solution was added to the each eppendorf tube. Then centrifugation was conducted at 10000 × g for 15 min at 4°C. After centrifugation supernatant was transferred to the new eppendorf tube. Then chloroform: isoamil solution treatment with subsequent centrifugation was repeated.

Then, supernatant was mixed with 60 µl 5М NaCl and 700 µl isopropanol, incubated in -200C for night. Incubated samples were centrifuged for 15 minutes at 4° C in 14000g. Extracted DNA was twice washed by 600 micro liters 70% ethanol and centrifuged for 5 minutes at 4° C in 10000g. After completing the final step of ethanol wash the DNA was left for air drying. Finally DNA was resuspended and dissolved in TE buffer and stored in -20° C.

DNA quantification and visualization. The DNA samples were diluted 20-25 times with ddH2O and quantified by taking the optical density (OD) at 260 and 280 with a spectrophotometer Cary 60 uv-vis(Agilent). 5µL DNA was run at 5 V/cm for 40 minutes on a 1% agarose gel and then photographed under UV illumination in the gel image analysis system.

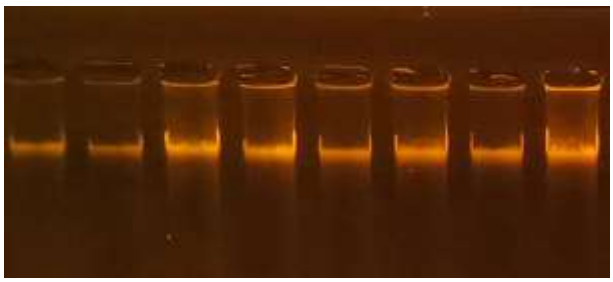

Fig. 2. Genomic DNA extracted using SDS method on 1% agarose gel

RESULTS AND DISCUSSION

In this study, we developed a simple, fast, and low-cost procedure to successfully extract genomic DNA from single aphids. The evaluation of integrity of the aphid genomic DNA isolated by this method showed intact/clear bands on agarose gel, suggesting that little or no DNA degradation had occurred during the extraction process (Figure 2). Spectrophotometer analysis showed that the A260/A280 ratios of DNA samples ranged from 1.67 to 1.94, suggesting that the DNA fraction was pure and may be used for further analysis. In all samples, the A260/A230 ratios were greater than 1.6, suggesting minimum contamination by polysaccharides. The DNA yields were from 97 to 150 ng/µL. The advantage of this method is its low cost. Grinding with liquid nitrogen and phenol treatment steps are eliminated using this methodology. All solutions used in this protocol are made from common and inexpensive chemicals.

It should be noted (due to pilot nature of our experiment) that there is one limitation of this study which. The fine quality of the genomic DNA isolated using this method and its applicability for subsequent molecular analysis needs to be further evaluated by PCR. Since DNA samples represents different species of cereal aphids, PCR should be conducted with universal invertebrate primers LCO-1490 and HCO-2198for amplification 658 base pair fragment of the 5' end of cytochrome c oxidase subunit I (COI) [10], which give PCR product for all species of aphids.

CONCLUSION

Despite this limitations, this method is an efficient, inexpensive, and reproducible technique to isolate total

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genomic DNA from aphid tissues, obtaining a good yield of DNA, even from very small specimens and this method has the potential to be useful for subsequent molecular genetic applications, and could represent a viable option for laboratories with low budgets.

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