



GENOME SIZE ESTIMATION OF POTATO APHID *MACROSIPHUM EUPHORBIAE* USING FLOW CYTOMETRY

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ABSTRACT

Potato aphid *Macrosiphum euphorbiae* (Thomas) (Hemiptera: Aphididae) is colonizing species and vector for many economically important potato viruses. There is dearth of genomic information about this economically important aphid species. Hence, to get insight into the genomic architecture, genome size was determined using flow cytometry. The estimated size of *M. euphorbiae* was 0.53 pg or 519.4 Mbp. The genome size of *M. euphorbiae* is approximately 2.9, 2.2 and 1.9x larger than that of *Drosophila melanogaster*, honey bee (*Apis mellifera*) and mosquito *Anopheles gambiae*, respectively. The generated genome size information will provide the foundation for futuristic genomic research on *M. euphorbiae*.

Key words: *Macrosiphum euphorbiae*, potato, flow cytometry, feulgen densitometry, genome size, mtCOI, *Drosophila melanogaster*, *Apis mellifera*, *Anopheles gambiae*

Potato aphid *Macrosiphum euphorbiae* (Thomas) (Hemiptera: Aphididae) is one of the colonizing aphid species on potato and vector of many economically important potato viruses (Fox et al., 2017; Xu and Gray, 2020). A number of virus diseases are spread by *M. euphorbiae*, among them *Potato Leaf Roll Virus* and *Potato Virus Y* are the predominant. They have complex lifecycles, comprising of both sexual and asexual (parthenogenetic) modes of reproduction. In addition to that it has been found that an aphid establishes complex relationships with their host plant and produce effectors that modulate host defense responses. The unusual biology of aphids makes them ideal models for the study of several biological processes that are not readily studied in other genetic model systems. In recent years, few studies have generated the genomic and transcriptomic data of aphid species (Czosnek and Ghanim, 2016; Teixeira et al., 2018; Chen et al., 2019) that has created genomic information which is now becoming useful for better understanding of aphid species. Being an important pest of potato with complex biology, *M. euphorbiae* has been studied at transcriptome level for identification of virus responsive genes and inhabiting plant viruses (Teixeira et al., 2018). Similar type of functional genomics studies are expected in near future. Hence, basic information about its genome size is crucial for various fields of research like evolutionary changes and taxonomic studies (Kron et al., 2007). Previously genome size of *M. euphorbiae*

was estimated to 0.40 pg using feulgen densitometry however, this method of genome size estimation has various drawbacks (Goldstein, 1981; Hardie et al., 2002). From last couple of years, flow cytometry has emerged as a significant method for DNA content analysis, as it is fast, convenient, and reliable. The determination of nuclear DNA amounts is performed with high precision using 1 to 5 % coefficients of variation (CV) in DNA peaks (Doležel et al., 2007). In this study, flow cytometry has been used for *M. euphorbiae* genome size estimation using external standard method.

MATERIALS AND METHODS

Adults of *M. euphorbiae* were collected from rose (*Rosa* spp.) plant grown in institute garden (CPRI, Shimla) at Shimla (31°5'14"N 77°11'6"E). Single parthenogenetic female was used to establish aphid colony on potato host and aphids from such colony was used for identification and genome estimation. Adults were collected in Falcon tubes (50 ml) for molecular identification as well as flow cytometry analysis. Species level identification was carried out by sequencing the Mitochondrial Cytochrome Oxidase-I (mtCOI) region using universal primer LCO1490/HCO2198 (Forward: 5'-GGTCAACAAATCATAAAGATATTG-3'; Reverse: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al., 1994). DNA of aphid was isolated using blood and tissue kit (Qiagen) following the

manufacturer guidelines and quantified using NanoDrop 2000/2000c spectrophotometer (Thermo Fisher Scientific). The PCR reaction consist of 10 μ l Emerald Amp GT master mix (2x), 1 μ M of each forward and reverse primer, 1 μ l of DNA templet (50 ng/ μ l) and the final volume of reaction was setup as 20 μ l with nuclease free H₂O. PCR was performed at 94°C for 4 min as initial denaturation, 35 cycles of 94°C for 30 sec, 50°C for 45 sec, 72°C for 1 min and a final extension was given with 72°C for 7 minutes. The visualization of PCR product was performed with 1% agarose gel and Qiaquick gel extraction kit (Qiagen) was used for purification. The PCR product after purification was cloned in ptz57R/T vector (thermos fisher scientific). Five positive clones were sequenced using genetic analyzer 3500 (ABI). Partial mtCOI sequences of *M. euphorbiae* were aligned in clustalW followed by construction of phylogenetic tree using Neighbor-Joining method (Saitou and Nei, 1987).

The samples for flow cytometry analysis were prepared as per the methodology given by the Doležel et al. (2007) with few modifications. About 10 adults *M. euphorbiae* were taken in 15 ml falcon and immersed in 1 ml of (modified hypopropidium iodide) HPI buffer. Tissues were homogenized in the 1 ml of modified HPI buffer (Krishnan et al., 1975) using the surgical blade. The homogenate was filtered through the 40-micron filters and incubated on ice under dark conditions with occasional shaking. Samples were analyzed on flow cytometer (BD FACS Canto II) by external standard method using chicken erythrocyte nuclei (CEN) (BD Biosciences, Cat No. 349523) as the external reference standard; and in three technical replicates, Data were recorded using the BD FACS Diva software. The genome size was estimated using the formula: $2C = 2.5 \times \text{mean position of sample nuclei peak} / 2C \text{ mean position of CEN nuclei peak}$

RESULTS AND DISCUSSION

The collected aphids were identified as *M. euphorbiae* based on multiple sequence alignment with reference sequence (Fig. 1). The mtCOI sequence reveals 100% similarity with reference sequences (Accessions no. MT651328.1, KY323034.1, KY323033.1 etc.) at NCBI database and one representative mtCOI sequence of *M. euphorbiae* has been submitted at NCBI wide accession no. MT821481. Mean peak position of G₀/G₁ cells was 13,784 and mean position of 2C peak of CEN was 63948 (Fig. 2). Based on this mean position, nuclear DNA content for *M. euphorbiae* was estimated to be

0.53 pg and in terms of base pairs it was estimated to be 519.4 Mbp. The estimated genome size slightly varied with previous reports, earlier it was estimated to be 0.40 pg using scanning micro-densitometry (Finston et al., 1995). It is reported that DNA amount has direct influence on duration of mitotic cycle and cell size, such phenotypic effects are called as ‘nucleotypic’ effects (Bennett, 1972) which denote the physico-mechanical properties of the nucleus, and it is assumed these can be attributed for slightly varied results. Many other factors can also affect genome size, such as accessory chromosomes fixation, polyploidy (Uozu et al., 1997; Ullmann et al., 2005), size of introns (Moriyama et al., 1998), transposable elements (Sanmiguel and Bennetzen, 1998; Vieira et al., 2002) and microsatellite presence (Warner and Noor, 2000) which needs to be instigated at cytological level. The genome size of *M. euphorbiae* (519.4 Megabase) is approximately 2.9x greater than that of *D. melanogaster* (176 Megabase) (Adams et al., 2000), 2.2x higher than that of honey

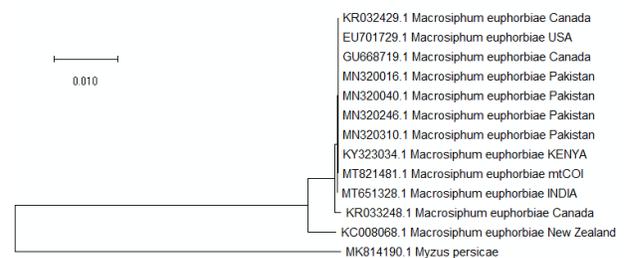


Fig. 1. Phylogenetic analysis of *M. euphorbiae* with reference sequences from NCBI. Multiple sequence alignment by clustal W followed by phylogenetic tree construction using Neighbor-Joining method with 1000 bootstrap value

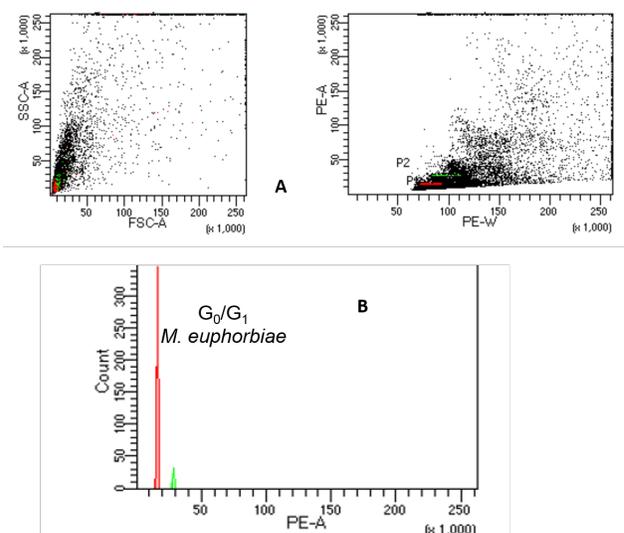


Fig. 2. The dot plot and bar graph of flow analysis; B, PI-fluorescence histogram of G₀/G₁ and G₂/M cells of *M. euphorbiae*.

bee (*Apis mellifera*) (234.7 Megabase) (Ardila-Garcia et al., 2010), 1.9x higher than that of mosquito *Anopheles gambiae* (264Megabase) (Holt et al., 2002), 1.4x that of the aphid *M. persicae* (Finston et al., 1995) and about equal in size of the *Bombyx mori* (508 Megabase) (Rasch, 1974). The flow cytometry-based determination of genome size of *M. euphorbiae* could serve foundation for whole-genome sequencing and shape sequence integrity.

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