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J. Environ. Biol.
33, 539-543 (2012)
ISSN: 0254-8704
CODEN: JEBIDP

Molecular typing of mealybug *Phenacoccus solenopsis* populations from different hosts and locations in Punjab, India

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Publication Data

Paper received:
26 June 2010

Revised received:
2 September 2011

Accepted:
20 September 2011

Abstract

True identity and existence of genetic variability in mealy bug from different regions holds immense significance for adopting appropriate control measures along with predicting the development of any biotypes. Mealy bug, *Phenacoccus solenopsis* adults were collected from four host plants i.e., *Gossypium hirsutum* – cotton (C), *Abelmoschus esculentus* – okra (O), *Pennisetum glaucum* – Napier Bajra (B) and a weed – *Parthenium hysterophorus* (P) in five cotton growing districts i.e., Abohar, Bathinda, Mansa, Muktsar and Faridkot of Punjab state. Variability among different populations was investigated through comparative analysis of four different RAPD markers. The genetic similarity dendrogram established that irrespective of the host plant and the collection site, 20 mealybug populations were distinguishable into two major clades that were related to each other by 68 %. Clade 1 included populations from Abohar district; it also included a single population each from Muktsar and Bathinda districts. The populations from all the other districts were grouped under Clade 2 with genetic similarity of 78 % Even under Clade 2, individual populations appeared to exist in location specific sub-clades. Thus, there is great possibility of development of biotypes which may differ in resistance to insecticides and host plant specificity.

Key words

Cotton, Genetic diversity, Mealy bug, *Phenacoccus solenopsis*

Introduction

Phenacoccus solenopsis (Tinsley) (Hemiptera: Pseudococcidae), (solenopsis mealybug) -originally described from the U.S. in 1898, has remained a serious pest of vegetable and floricultural crops in Central America, Caribbean islands, and Ecuador (Williams and Willink, 1992). Subsequently, this pest was reported from Chile and Brazil on tomato and several weed plants (Culik and Gullan, 2005) indicating that weeds in the neighborhood fields may act as reservoir for this pest. Low incidence of mealy bug has also been recorded on cotton crop in India (Muralidharan and Badaya, 2000). A severe incidence was reported from Pakistan in 2005 (Abbas *et al.*, 2005) and from Ferozepur, Abohar, Muktsar and Bathinda districts of Punjab (India) during 2006, and subsequently spread to other districts in 2007. In 2007-08, the cotton belt of Punjab was severely threatened by *P. solenopsis* causing huge losses to the cotton crop (Dhawan *et al.*, 2007). Both adults and crawlers suck the sap from the stems, twigs, leaves, flower buds and young bolls. Feeding of mealy bug causes premature leaf drop, dieback and may even kill plants if left untreated. Besides, honeydew a sugary liquid is excreted by these insects on the leaves and serves as a medium for the growth of sooty mold fungus that reduces photosynthetic abilities (Saeed *et al.*, 2007). A

severe infestation results in bunchy growth, stunting, reduction in boll formation and boll size, which ultimately reduces the overall yield (Ben-Dov, 1994) The damage of *Phenacoccus solenopsis* initiates from the border and then spread to the entire field. Besides cotton, *Phenacoccus solenopsis* was also observed on weeds, viz, *Parthenium hysterophorus* (most preferred), *Abutilon* sp., *Tribulus terrestris*, *Achyranthes aspera Trianthema monogyna, Calotropis procera, Digera arvensis*, *Cynodon dactylon, Amaranthus viridis*, *Acrachne racemosa* and other crops like *Cucumis* sp., *Abelmoschus esculentus, Sesbania sesban*, ornamental plants like *Hibiscus* sp. and *Chrysanthemum* sp. and also on trees of *Psidium guajava*, zizypus and *Ficus religiosa*. This mealy bug feeds on around 300 crops on the subcontinent (Ashfaq *et al*., 2010). The problem with mealy bug has spurred the search for effective and quick control measures through application of different insecticides (Mohyuddin *et al*., 1997). Wide host range of the pest may facilitate the development of new biotypes which may be difficult to control with a single strategy. Besides the true identity of this pest species, exploring the existence of genetic variability from different regions holds immense significance for adopting appropriate control measures.

RAPD-PCR is conceptually simple technique for estimation of genetic diversity of organism (Weish and McClelland, 1990). The RAPD technique has been widely used to elucidate the geographical origin, host specificity and gene flow among insect populations (Vandewoestijne and Baguette, 2002; Ayres *et al.*, 2003; Williams *et al.*, 1994; Tabemer *et al.*, 1997; Bas *et al.*, 2000; Scataglini *et al*., 2000). Limitations associated with RAPD markers, like variable reproducibility, may appear unless reaction conditions are stringently controlled and a dominant mode of inheritance is lacking (Black, 1993; Lynch and Milligan, 1994; Loxdale *et al.*, 1996). Nevertheless, properly performed RAPD analysis is a useful and reliable tool for studying the ecology and genetic structuring of many species populations (Armstrong and Wratten, 1996; Brown *et al*., 1997; Vaughn and Antolin, 1998; Pearson *et al*., 2002). Therefore, in the present investigation we evaluated the genetic diversity among *P. solenopsis* populations collected from different cotton growing areas and from different host plants in the state of Punjab.

Materials and Methods

Adults of mealybug *(Phenacoccus solenopsis)* were collected from four host plants i.e., *Gossypium hirsutum* – cotton (C), *Abelmoschus esculentus* – okra (O), *Pennisetum glaucum* – napier bajra (B) and a weed – *Parthenium hysterophorus* (P) growing in fields of five different cotton growing districts, (Abohar (Abo), Bathinda (Bat), Mansa (Man), Muktsar (Muk) and Faridkot (Far)) of Punjab, during November-December 2007. The adults were stode in 95 % ethanol. Total DNA was isolated from five adults population by CTAB method (Phillips and Simon, 1995) and ultimately suspended in 100 µl of Tris-EDTA buffer. Any contamination with RNA was removed by including RNAse enzyme (100µg ml^{-1}) in Tris-EDTA buffer itself. The DNA concentration in final suspension (~ 10 µg ml^{-1}) was quantified by absorbance at A_{260} and gel electrophoresis.

Molecular markers specific to *P. solenopsis* are not available, and hence genetic variability amongst different populations was investigated through comparative analysis of different RAPD (Random Amplified Polymorphic DNA) markers and RAPD-PCR amplification profiles. For this purpose, total DNA from adults of each population was PCR amplified using 12 different RAPD primers (OPH-16, OPB-05, OPB-7, OPB 12, OPB 17, OPE-03, OPF-02, OPF-16 and OPC-04, OPC 11, OPH 9, OPH 16) (Operon-America). However only 4 primers viz. OPH 16, OPB 05, OPF-02 and OPC-04 generated polymorphic bands and were subsequently used for calculating the similarity coefficient. The PCR amplifications were performed in 25 ml reaction mixture, which contained 1X Taq reaction buffer, 0.2 mM dNTPs mix, 0.4 mM of RAPD primer, 2.4 U Taq Polymerase (MBI, Fermentas) and 3ml (~30 ng) of template DNA. RAPD- amplifications were performed in a PTC-100 thermal cycler (MJ research) using a PCR program of 95ºC- 5'; 95ºC- 1', 40ºC- 1', 72ºC- 2' (39 cycles); 72ºC- 10'. The amplified bands were separated by agarose (1.5 per cent) gel electrophoresis in TAE (Tris base, Acetic acid and EDTA-Ethylene diamine tetra acetic acid) buffer and visualized in ethidium bromide stained gel on UV-transilluminator. The size of individual DNA fragments were compared with a co-migrating 100 bp DNA ladder (MBI Fermentas). In order to quantify the same, similarity matrices were derived from the data based on presence (1) or absence (0) of all the different bands that were amplified for each of mealybug population from different locations/ host plants. On the basis of 'F estimator of similarity between pairs' of mealybug samples, a dendrogram was generated using 'SimQual' function of the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method of the program NTSYS pc - version 2.0.

Results and Discussion

RAPD-PCR of different *P. solenopsis* populations with four different RAPD primers generated a similar banding profile, however some polymorphic markers were also identified, indicating genetic variability among mealybug populations (Fig 1). The 500 bp band was present in all the almost all the populations, however this was completely absent in the Mansa-Okra and less conspicuous in Muktsar-Parthenium and Bathinda-Bajra. Similarly, a band of ~ 250bp was present in all the populations except Abohar-Okra and Mansa-Okra. A ~ 700 bp band was present in all the Bathinda population except in Bathinda-Okra. Such many polymorphic bands were present in one or the other populations and have been marked with black pointer (▶) in Fig. 1. These polymorphic bands amplified with different primers contributed towards calculating the genetic variability among the various mealybug populations that has been reflected through dendrogram (Fig 2) . The genetic similarity dendrogram established that irrespective of the host plant and the location of collection, 20 mealybug populations were distinguishable into two major clades that were related to each other by 68 per cent (Fig 2). Whereas Clade 1 included populations from Abohar district,

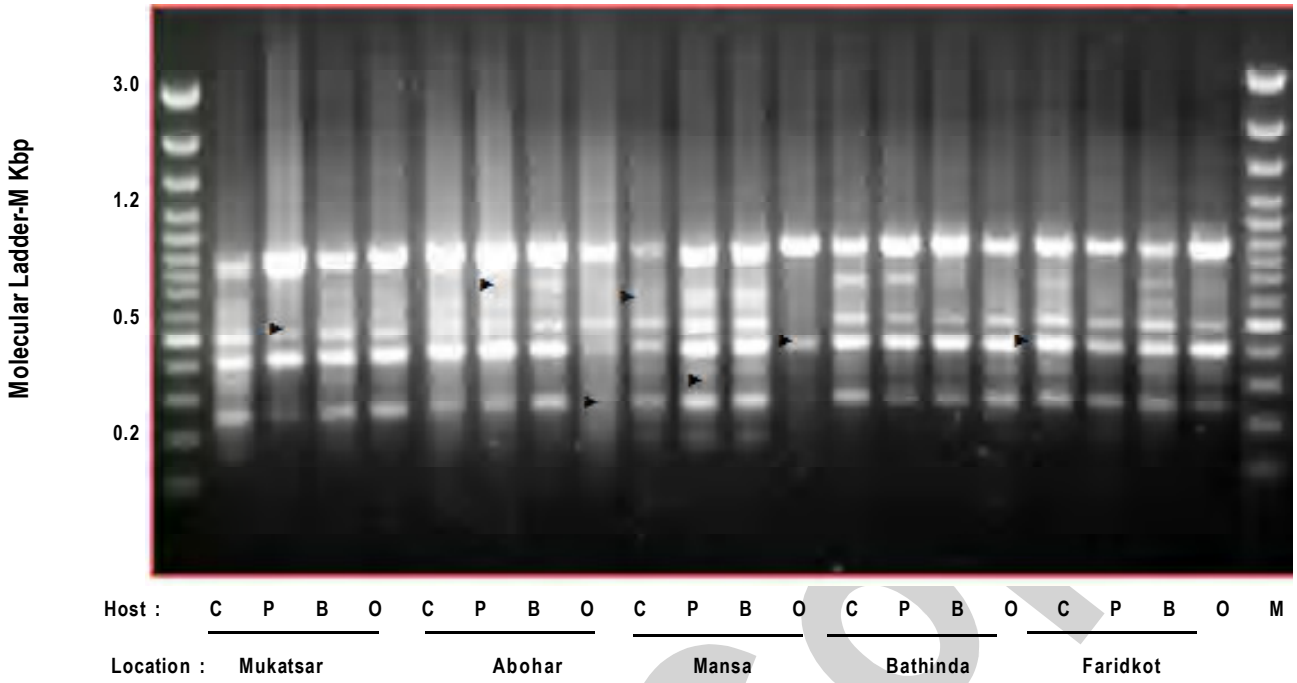

Fig .1: Comparative RAPD- amplification profile of *Phenacoccos solenopsis* populations from different plant hosts and locations of Punjab state with RAPD primer OPB-05. Host crop: C- Cotton, P- Parthenium, B- Napier Bajra, O- Okra. ▶ - Polymorphic marker bands: M- 100 bp DNA ladder (kbp).

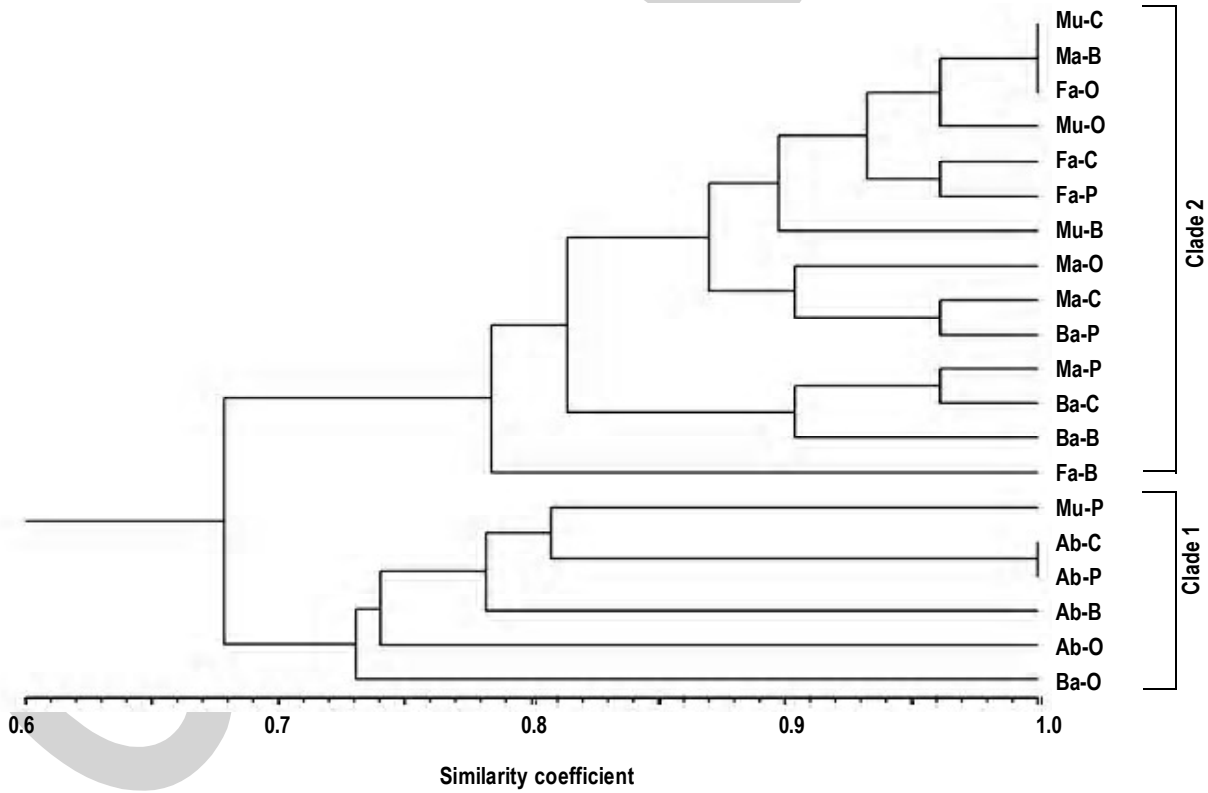

Fig .2: Dendrogram showing genetic similarity among different populations of mealybug. Host crops: C- Cotton, P- Parthenium, B- Napier Bajra, O- Okra. Locations: Ab- Abohar, Ba- Bathinda, Fa- Faridkot, Ma- Mansa, Mu- Muktsar.

it also included single population each from Muktsar and Bathinda districts on parthenium and okra, respectively. The populations from all the other districts were grouped under Clade 2 with genetic similarity of 78 per cent. Even under Clade 2, individual populations appeared to exist in location specific sub-clades. However, mixing of populations from different locations in some of sub-clades could be attributed to proximity of the locations from each other in the cotton growing region of Punjab. As *Parthenium* is the major host contributing towards off-season and in-season survival of mealybug (Arif *et al.,* 2009). The bugs surviving on *Parthenium* weeds contribute the major source of inoculum for the cotton crop (Abbas *et al,* 2010). The dendrogram clearly indicates close proximity of populations from cotton and *Parthenium*. The population from Faridkot-cotton (Fa-C) and Faridkot-*Parthenium* (Fa-P) show 96% similarity, similarly the Abohar-cotton (Ab-C) and Abohar-*Parthenium* (Ab-P) populations appear to be the same showing 100% similarity and the populations from two close regions i.e Mansa-cotton (Ma-C) and Bathinda-*Parthenium* (Ba-P) and vice-versa share 96% similarity between each other and 81% among themselves. It is likely that cotton mealybug was introduced in India from Pakistan where it appeared in 2005 (Abbas *et al.,* 2005), and subsequently reported from border district of Ferozepur (Abohar) in 2006. Consequently, we observed that all the four populations from Abohar (Ab-C, Ab-P, Ab-B and Ab-O) fall in the Clade 1 and share similarity of 74% This might be indicative that this group might be representing the one from Pakistan. The Clade 2 represents the populations from the interiors and it is most likely that these have populations have originated from clade1 and subjected to slight changes due to host and environmental selection pressure. RAPD markers (8 in number) identified specificity in *Bemisia tabaci* (Genn.) to a single or two different host plants (cotton, brinjal, tomato, soybean and *Sida* sp., a weed) (Gupta *et al,* 2010). Host plant was associated with the influence on biology of *P. solenopsis.* Developmental periods for the 1st, 2nd and 3rd instars *on H. rosa-sinensis* were found progressively increasing to 6, 8 and 10 days, respectively; however *P. solenopsis* under laboratory conditions had longer developmental periods for the 2nd instar over the other two instars (Akintola and Ande, 2008). Thus, it may be possible that if host can influence the biology of pest consequently it may also be associated with molecular changes. The results establish that mealybug populations in Punjab state represent a vast genetic variability. Thus, there is great possibility of development of biotypes which may differ in resistance to insecticides and host plant specificity (Diehl and Bush, 1984). This also stresses upon the need for adoption of different control measures strategies based upon existing genetic diversity in the solenopsis mealybug populations such as eradication of weed hosts such as parthenium and discouraging growing of okra around the cotton fields. RAPD-PCR has proved to be an appropriate method for obtaining genetic markers for many different kinds of organisms including insects. (Kim and Sappington, 2004). Thus, the information generated can be used for future programmes for the development specific markers for easy identification of geographical/ host specific strains of the pest.

Acknowledgement

Authors are grateful to the Head, Department of Entomology, Punjab Agricultural University, Ludhiana for providing necessary facilities.

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