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ISSR-PCR: Tool for discrimination and genetic structure analysis of *Plutella xylostella* populations native to different geographical areas ☆

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Abstract

The diamondback moth (DBM), *Plutella xylostella* (L.) is considered as the most destructive pest of Brassicaceae crops world-wide. Its migratory capacities and development of insecticide resistance in many populations leads to more difficulties for population management. To control movement of populations and apparitions of resistance carried by resistant migrant individuals, populations must be identified using genetic markers. Here, seven different ISSR markers have been tested as a tool for population discrimination and genetic variations among 19 DBM populations from Canada, USA, Brazil, Martinique Island, France, Romania, Austria, Uzbekistan, Egypt, Benin, South Africa, Réunion Island, Hong Kong, Laos, Japan and four localities in Australia were assessed. Two classification methods were tested and compared: a common method of genetic distance analyses and a novel method based on an advanced statistical method of the Artificial Neural Networks' family, the Self-Organizing Map (SOM). The 188 loci selected revealed a very high variability between populations with a total polymorphism of 100% and a global coefficient of gene differentiation estimated by the Nei's index (*Gst*) of 0.238. Nevertheless, the largest part of variability was expressed among individuals within populations (AMOVA: 73.71% and mean polymorphism of 94% within populations). Genetic differentiation among the DBM populations did not reflect geographical distances between them. The two classification methods have given excellent results with less than 1.3% of misclassified individuals. The origin of the high genetic differentiation and efficiency of the two classification methods are discussed.

Keywords: Decision-making tool; Inter Simple Sequence Repeat; Genetic differentiation; *Plutella xylostella*; Self-Organising Map; Pest management; Population analysis

1. Introduction

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera; Plutellidae), is the major cosmopolitan pest of brassica and other crucifer crops in many areas of the world. DBM can live under wide climatic conditions and is known to migrate across the world (Chu, 1986; Honda, 1990; Honda et al., 1992; Chapman et al., 2002; Coulson et al., 2002). DBM is a prolific species in tropical climates, where it can have more than 20 generations a year. DBM can cause more than 90% crop loss (Verkerk and Wright, 1996) and only few fourth stage larvae on a cabbage can make it unsaleable (Shelton et al., 1983; Maltais et al., 1998).

Extensive insecticide applications are used for its management which has led to a rapid increase in DBM resistance. Resistance to DDT appeared in 1953 (Ankersmit, 1953) and to *Bt* in 1980 (Tabashnik et al., 1990; Shelton et al., 1993a,b; Tabashnik, 1994). The cost of DBM populations control worldwide has been estimated as

☆ This study forms a part of O. Roux's PhD thesis on the relationship between DBM and a larval parasitoid *Cotesia plutellae*. The authors are part of a university laboratory which has for interest diversity and evolution in agro-ecosystem and part of an Agricultural Research Centre for International Development where some laboratories are implicated in IPM in tropical areas. M. Gevrey was developing and adapting neural network to molecular work. L. Arvanitakis and D. Bordat are IPM entomologists were providing us the background of knowledge on DBM, reared in laboratory all populations from fields. This work was directed by L. Legal and C. Gers who are both evolutionary ecologists.

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approximately one billion US $ annually (Talekar and Shelton, 1993).

Many authors have noticed differences in susceptibility to many insecticides between DBM strains (Díaz-Gomez et al., 2000; Gonzalez-Cabrera et al., 2001; Mohan and Gujar, 2002, 2003; Liu et al., 2003). However, migration capabilities of the pest cause difficulties for strains determination and delay the use of IPM programs. The development of some markers is necessary to identify and characterize DBM strains. Such markers have to be low cost and to give fast results.

This topic study explores the usefulness of Inter Simple Sequence Repeat (ISSR) markers to identify and discriminate several populations of DBM worldwide through genetic variations. The ISSR is known to evolve rapidly and consequently generate a large number of polymorphic bands at the intraspecific level. Bands are generated by a single-primer PCR reaction where the primer is a repetition of a di-, tri- or tetranucleotide and the amplified region is a portion of genome between two identical microsatellite primers with an opposite orientation on the DNA strand. These primer sequences are broadly distributed on the genome. Therefore, the ISSR-PCR technique permits to screen quickly a wide part of the genome without prior DNA sequence knowledge. As for RAPD (Random Amplification of Polymorphic DNA), ISSR bands are considered as dominant markers but have higher reproducibility (Fang and Roose, 1997; Nagaoka and Ogihara, 1997). The diallelique interpretation (presence/absence) may cause matters. Indeed the absence of a band can correspond to one or several divergences in primer site or to a chromosomal rearrangement (Wolfe and Liston, 1998) and presence of two bands with the same weight does not necessarily affirm similarity, as the variability is probably underestimated. Nevertheless, ISSR has already been used in numerous organisms for genetic characterization (Reddy et al., 1999; Sobhian et al., 2003; Cano et al., 2005), to assess genetic diversity (Qiu et al., 2004; Wang et al., 2005; Lu et al., 2006; Zhang et al., 2006), to identify genetic trait loci (Zietkiewicz et al., 1994; Ratnaparkhe et al., 1998; Blair et al., 1999; Arcade et al., 2000), and for understanding phylogenetic and/or interspecific relationships (Wolfe and Liston, 1998; Josh et al., 2000; Wolfe and Randle, 2001; Datwyler and Wolfe, 2004; Wu et al., 2005).

Several families of Lepidoptera have been investigated using ISSR markers; Noctuidae, Pyralidae, Pieridae and Sphingidae (Luque et al., 2002; Hundsdoerfer et al., 2005; Hundsdoerfer and Wink, 2005). An interesting element is that depending the type of population studied (open or close), variability and number of informative bands varies from 50% for the localized species: *Diarsia brunnea* (Noctuidae) (Luque et al., 2002) to 100% for a quasi cosmopolitan species such as *Pieris rapae* (Pieridae) (Hundsdoerfer and Wink, 2005).

The Self-Organizing Map (SOM) (Kohonen, 1982), which is an advanced statistical method of Artificial Neural Networks' family, is an efficient method when complex non-linear relationships are present in the analysed system to classify complex data (Lek et al., 1996; Lek and Guégan, 2000; Park et al., 2003a). SOM provides an alternative to traditional statistical methods as Principal Component Analysis, Polar Ordination, Correspondence Analysis and Multidimensional Scaling (Foody, 1999; Giraudel and Lek, 2001; Brosse et al., 2001). SOM's are used widely for knowledge discovery, pattern recognition, clustering and visualisation of large multi-dimensional datasets (Ferran and Ferrara, 1992; Chon et al., 1996; Park et al., 2003b; Gevrey et al., 2004). To our knowledge only a few recent studies have used SOM with genetic data (Giraudel et al., 2000; Zhao et al., 2006), but it has been successfully used over the last few decades in biology (Lek and Guégan, 2000; Recknagel, 2003; Lek et al., 2005). This study attempts to associate a molecular marker and a classification statistical method to provide a complete decision-making tool in DBM invasion management.

2. Materials and methods

2.1. Plants and insects

DBM populations native to 16 countries and 19 different localities given in Table 1 were collected on cabbage, *Brassica oleracea* var. *capitata*. DBM females laid on Indian mustard, *Brassica juncea*, in Plexiglas $50 \times 50 \times 50$ cm cages. Water and honey were provided as food *ad libitum*. All larval stages were reared on *B. oleracea* var. *capitata*. DBM populations were maintained in climatic rooms at 25 ± 1 °C, 40–50% RH and a 12L:12D photoperiod. All adults used to this study were spring from the first rearing generation.

For molecular analysis, DBM adults were killed in liquid nitrogen and conserved to −80 °C until DNA extraction.

2.2. DNA extraction

Abdomens were cut from dead and frozen DBM males and incubated 12 h at 50 °C in 350 μL of lyses buffer B (10 mM Tris, pH 7.5, 25mM EDTA, and 75 mM NaCl) with 500 μg of Proteinase K and 20 μL of 20% SDS. Proteins and residues were precipitated with 200 μL of saturated NaCl solution and centrifuged at 1400 rpm for 30 min. DNA from supernatant was saved and precipitated with 400 μL of cold isopropanol and centrifuged at 1400 rpm for 40 min at 2 °C. The isopropanol was eliminated and the precipitate was washed with 500 μL of 70% ethanol, centrifugated at 1400 rpm for 10 min at 2 °C, dried and redissolved in 100 μL of TE buffer and conserved at −28 °C until utilization.

2.3. ISSR-PCR and electrophoresis

Inter Simple Sequence Repeat (ISSR) analysis was performed using seven different primers listed in Table 2.