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COMPARATIVE STUDY ON ESTERASE ISOZYME PATTERNS IN THE ADULT FLY OF Bactrocera papayae AND B. carambolae (Diptera: Tephritidae)

M. Hasanuzzaman and Idris A.B.
School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia.
E-mail: mhasanuzzaman72@yahoo.com

INTRODUCTION

Asian papaya fruit fly, Bactrocera papayae Drew & Hancock and carambola fruit fly, Bactrocera carambolae Drew & Hackcock (Diptera: Tephritidae) are the major agricultural pests, especially fruits and vegetables in Malaysia. Its presence can cause severe damage of the agricultural produce as well as limitations to the import/export of these products. Esterase comprises a multi-functional and heterogeneous group of enzymes which have as a shared characteristic participation in ester hydrolysis. In insects, they are related to several metabolic processes, such as food digestion, degradation of insecticides/insecticide resistance, pheromones and juvenile hormone hydrolysis (Campbell et al., 2003). Esterase patterns are important tool for analysis of genetic differentiation and evolutionary relationship of insects (Nascimento and de Campos Bicudo, 2002). They are also stage-specific and tissue-specific in insects (Lima-Catelani et al., 2004) and are closely associated with morphological, physiological, or biochemical ontogenetic alterations (Cohen et al., 1977). A polyacrylamide gel electrophoretic technique was used to study the esterase isozyme patterns in the adult flies of these two Bactrocera species. The results prove that different species have different esterase bands on gel. This technique can be used to distinguish the adults of the two species.

MATERIALS AND METHODS

Insect culture: Initially the pupae of B. papayae and B. carambolae were collected from Malaysian Agricultural Research and Development Institute (MARDI). Then the culture has been maintained generation wise in the laboratory, Universiti Kebangsaan Malaysia. Mixture of yeast and sugar (1:3) served as adult diet and water was supplied through soaked cotton. Fresh star fruits were used for egg laying as well as larval media. Rearing was maintained at 25±2°C with 70-80% relative humidity and 14h light: 12h dark cycle.

Sample preparations for electrophoresis: Adults of B. papayae and B. carambolae from the laboratory reared stock were used for the experiments. According to Bernardo and de Campos Bicudo (2009), two individuals of each stage were homogenized for a better visualization of the bands in the gel, at 0°C in 25 μl of buffer solution (0.1 M Tris-HCl plus 10% glycerol at pH 8.8). Ten micro liter of bromophenol blue (0.05 mg/ml) was added into each sample as a tracking dye. Homogenates were centrifuged at 10,000 rpm for 15 min at 5°C.

Gel preparation and running of the gels: Esterase patterns were analyzed in polyacrylamide gels using a 10% separating gel and a 4% stacking gel (Laemmli, 1970). After the sample application (10 μl), the gels were subjected to electrophoresis for 4 h at room temperature (25°C) using a constant voltage of 200 V and 0.1 M Tris-glycine (pH 8.3) as the running buffer (Bernardo and de Campos Bicudo, 2009).

Identification of esterases in the gels: Esterases were identified in the gels following the technique described by Johnson et al. (1966); Steiner and Johnson (1973), using α- and β-Naphthyl acetates as substrates.
Numbering of esterase isozymes: The esterase isozyme bands were numbered from the anodal end of the gel according to the recommendations of the Standard Committee of Enzyme (Webb, 1964).

Measurements of relative mobility (Rm): The relative mobility (Rm) value of esterase isozymes was calculated using the following formula (Raja et al., 2009).

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R_m = \frac{\text{Distance of isoenzyme migration (cm)}}{\text{Length of gel after staining (cm)}} \times \frac{\text{Length of gel before staining (cm)}}{\text{Distance of dye migration (cm)}}
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RESULTS AND DISCUSSION

The electrophoretic banding patterns of nonspecific esterase isozymes were observed on Polyacrylamide Gel Electrophoresis (PAGE) in the male and female adult flies of Bactrocera papayae and B. caramboiae (Diptera: Tephritidae). Esterase isozyme patterns were shown in Fig. 1. Two esterase isozymes, EST-1, EST-2 were detected and their relative mobility values were 0.46 and 0.15, respectively. EST-1\(^{0.46}\) was highest mobility and EST-2\(^{0.15}\) was lowest mobility and close to the cathode. Two bands, EST-1\(^{0.46}\) and EST-2\(^{0.15}\) were present in B. caramboiae, whereas only one band, EST-2\(^{0.15}\) was observed in B. papayae. The thickness and the staining degree of bands varied in the adult flies of these two species. This observation is important because high degrees of staining and thickness are indicative of great enzymatic activity. EST-2\(^{0.15}\) band was thick and highly stained than the band of EST-1\(^{0.46}\) in adult flies of B. caramboiae. There was no significant difference in male and female adults, when comparing among the same species. Several works were done for the esterase isozymes banding patterns in insects. Borja et al. (2010) found four esterase isozyme bands, controlled by two loci (EST-1, EST-2) in Bactrocera occipitales and B. philippinensis. Hasanuzzaman (2003) reported seven esterase bands.

controlled by two esterase loci (EST-1 and EST-2) during the different life stages of Bactrocera cucurbitae on Polyacrylamide Gel (PAGE) and their relative mobility values were 0.17, 0.27, 0.37, 0.46, 0.58, 0.87, 1.00. Two esterase loci (EST-1 and EST-2) were detected in the adult brown planthopper, Nilaparvata lugens by Bashar et al. (2002) in PAGE gel. Cohen et al. (1977) studied the expression of esterases during ontogeny of the flour beetle Tribolium castaneum in PAGE gel. Two nonspecific esterases were detected and designated F (fast) and S (slow) according to their relative migration distances. In the adults of Drosophila aurilis, the maximum activity/intensity of esterase patterns was detected by Sasaki (1974). In conclusion, the results of the present research can be used for identifying these pests and also strengthen the necessity for a comparative study between other Bactrocera species in Malaysia and may impose a significant impact on the tropical environment.
REFERENCES


