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HOST ASSOCIATED VARIATIONS IN ESTERASE AND GLUTATHIONE-S- TRANSFERASE IN *BEMISIA TABACI*

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ABSTRACT

In the present study, the populations of *Bemisia tabaci* were collected from five locations on cotton and subsequently reared and established on brinjal and tomato to study host associated variations in esterases and glutathione-s-transferase. Pyrethroids, carbamates and organophosphates were the most widely used group of insecticides in these locations.Frequent failure of these conventional pesticide sprays have been continuously reported that may be attributed to enhanced levels of detoxifying enzymes due to continuous selection pressure. Significant variation in both the enzymes levels was found across the population and host. Khandwa population recorded the highest esterase activity with 2.21 fold (4.33± 0.48 mOD/min/0.5 *B. tabaci* equivalent), 1.67 fold (4.83± 0.39mOD/min/0.5 *B. tabaci* equivalent) and 1.69 fold (5.34± 0.38 mOD/min/0.5 *B. tabaci* equivalent) for brinjal, cotton and tomato respectively.Population from Khandwa and Sriganganagar recorded the highest activity of glutathione-s-transferase with 1.71 folds (243.77± 6.56nmol/min/*B. tabaci* equivalent, 243.88± 25.66nmol/min/*B. tabaci* equivalent) for both population on brinjal; 1.75 (263.53± 17.53nmol/min/*B. tabaci* equivalent) and 1.76 (265.74± 18.04nmol/min/*B. tabaci* equivalent) folds on cotton; 1.72 (289.28± 9.82nmol/min/*B. tabaci* equivalent) and 1.66 (279.40± 23.21nmol/min/*B. tabaci* equivalent) folds on tomato as hosts respectively taking New Delhi population as unity. Tomato recorded the highest value for both the enzymes followed by cotton and brinjal as host.

Key words: *Bemisia tabaci*, cotton, brinjal, tomato, detoxifying enzymes, esterase, glutathione-S-transferase, resistance

Whitefly *Bemisiatabaci* (Gennadius) (Hemiptera: Aleyrodidae), is considered to be most devastating pest of global significance affecting field, ornamental and vegetable crops causing severe economic losses. The wide scale adaptation of this pest can be attributed to development of resistance to many classes of insecticides coupled with its polyphagous naturewith more than 600 documented host plants. Both these factors make this pest as an excellent representative for studying host plant interactions with detoxifying enzymes (Oliveira et al., 2001).After introduction of *Bt* cotton in 2002, pest scenario changed considerably bringing bollworms under check with serious incidences of sucking pests like whiteflies. It led to severe crop losses caused through direct feeding, excretion of honeydew, and transmission of plant viruses (Jones, 2003). Due to its extensive damage, it is predominantly controlled with insecticides leadingto evolution of resistance (Roush and Tabashnik, 1990). Insecticide resistance involves three major mechanisms, decreased penetration, enhanced detoxification, and target site insensitivity. Among these, detoxifying enzymes play important role in imparting insecticide resistance (Owusu et al., 1995; Li et al., 2007; Alon et al., 2008) and host plants are known to affect the activity and degree of expression of these enzymes in insects (Liang et al., 2007, Wang et al., 2010; Xue et al., 2010; Xie et al., 2011). Further, there are reports about the responses of the herbivore, which not only changes between the host plants species, but also intra specific variations are being reported within varieties of the same crop (Iida et al., 2009; Xie et al., 2011;Yan et al., 2011).

In the present study, the populations of *B. tabaci* were collected from five locations on cotton in 2014. These populations were subsequently reared for 6-8 generations on brinjal and tomato and established population were used to study host associated variations for esterases and glutathione-s-transferase. As per the information collected from farmers during survey, although recommended doses of organophosphates, carbamates and pyrethroids were sprayed, the control was not up to the markthat may be due to indiscriminate use of pesticides in previous years. Reduced susceptibility can be ascribed to enhanced

levels of major detoxification enzymes like esterases and glutathione s-transferase.

MATERIALS AND METHODS

The population of *B. tabaci* were collected from cotton crop of five locations falling under two major cotton growing regions of India [North region: Sriganganagar (Rajasthan, 29°55'12" N; 73°52'48" E), Ludhiana (Punjab, 30°36'0.338" N; 74°47'41.719" E) and Delhi (28°38'5.940" N; 77°09'6.750" E); Central region; Khandwa (Madhya Pradesh, 21°48'56" N; 76°22'11" E) and Amravati (Maharashtra, 20°58'715"N; 77°46'437" E)].The collections were made in August-September months in 2014. While collecting, Zee walk mode was followed. Adult whiteflies were collected early in the morning using an aspirator; green leaves were plucked to collect immature stages. The insects were transferred in ventilated cages containing leaflets inserted into wet sponges. Collected populations were kept in rearing cages for the emergence of fresh adults and the 'puparia' were sampled for valid species authentication (Martin, 1987). The respective populations were raised in insecticide free exposure conditions on cotton (*Gossypium hirsutum* L), brinjal (*Solanum melongena* L), tomato (*Lycopersiconesculentum* Mill) seedlingsfor 6-8 generations, at 27± 2°C temperature, 14:10 h (L:D) photoperiod and 60-70% relative humidity in insect rearing chamber at Division of Entomology, ICAR-IARI, New Delhi. Host established populations were used to study host associated variations for esterases and glutathione-s-transferase.

The esterase activity was estimated by microplate assay using α–naphthyl acetate as substrate (Alon et al., 2008; Stumpf and Nauen, 2002). The esterases catalyse the hydrolysis of carboxylic esters to free acid and alcohol. It can be measured by the product formed from substrate α–naphthyl acetate to α–naphthol.The adults were taken from the population established in the laboratory. The homogenates of five adult flies was prepared using a hand held homogenizer with a plastic pestle (Sigma-Aldrich) in 250 µl ice-cold sodium phosphate buffer (0.1 M, pH 7.5), containing 0.1% (w/v) Triton X-100. The homogenate was short spun and used as enzyme source. The esterase activity was measured as per standard protocol (Shah et al., 2017) and expressed in mOD/min/0.5 *B. tabaci* equivalent.

The total GST activity was measured through the conjugation of CDNB with reduced GSH (Habig et al., 1974). The conjugation results in increased absorbance at 340 nm. The rate of increased absorbance is related to the GST activity in the sample.Enzyme source for GST assay was prepared from 50 adults homogenized using hand held homogenizer with a plastic pestle in 100 µl sodium phosphate buffer (0.2 M, pH 7.5 contained 2mM EDTA). The homogenates were then centrifuged at 12000 g for 10 min at 4°C and the resulting supernatant was used as enzyme source. The activity was measured per standard protocol (Shah et al., 2017) and expressed in nmol/min/*B. tabaci* equivalent. Activities of esterases and glutathione-s-transferase among the populations were compared using Tukey's test ($p<0.10$).

RESULTS AND DISCUSSION

A significant variation in esterase and glutathione-s-transferase was observed across host plants and populations of *B. tabaci*. These two enzymes play a major role in detoxification of most of the conventionally used group of insecticides. Variations in these enzymes in the five field population of *B. tabaci* was studied and compared with New Delhi population (lowest enzyme activity) that was taken as unity to compare fold increase in enzyme activity.

A greenhouse experiment was conducted for executinginter-species transfer of *B. tabaci* population on four host plants. Population from cotton and cucumber had significantly higher enzyme activity when compared with that of pumpkin and vegetable marrow (Liang et al., 2007). In the present study, among *B. tabaci* population reared on three host plants (brinjal, cotton and tomato) brinjal recorded the least value for esterase activity followed by cotton and tomato (**Table** 1). Studies on enzyme activity in relation to host preference of *B. tabaci* has revealed that high preferred host usually show low enzyme activity and shift to low preference host increases the activity (Zhou et al., 2010). In the present study, brinjal had least enzyme activity indicating its preference among three hosts.

Among locations, Khandwa population recorded the highest esterase activity with 2.21 fold (4.33± 0.48 mOD/min/0.5 *B. tabaci* equivalent), 1.67 fold (4.83± 0.39 mOD/min/0.5 *B. tabaci* equivalent)and 1.69 fold (5.34± 0.38 mOD/min/0.5 *B. tabaci* equivalent) for brinjal, cotton and tomato respectively. Population from Amravati recorded values with 1.74, 1.61 and 1.64 fold increase for brinjal, cotton and tomato respectively compared to New Delhi population. The fold increase in esterase activity of Sriganganagar and Ludhiana population was almost on par with a fold increase of 1.45 and 1.37 on brinjal, 1.13 and 1.15 on cotton, 1.56 and 1.58 on tomato respectively. New Delhi population

recorded the least esterase values with 1.96± 0.13 mOD/min/0.5 *B. tabaci* equivalent, 2.90± 0.23 mOD/min/0.5 *B. tabaci* equivalent and 3.17± 0.28mOD/min/0.5 *B. tabaci* equivalent for brinjal, cotton and tomato respectively (Table 1). The esterase values were significantly higher in tomato as host compared to cotton and brinjal in all the populations evaluated except Khandwa, where no such significant difference across the hosts was recorded. This population also had highest enzyme activity.

Expression of GST genes increased 1.7 to 2.74 folds when adults of *B. tabaci* were transferred to cabbage or mustard from cotton. Subsequently, when adults were brought back to cotton, corresponding decrease in genes expression levels was observed implicating the role of host plant in governing the levels of detoxifying enzymes (Morin et al., 2008). In the present investigation, activities of GST in population of *B. tabaci* reared on three host plants (brinjal, cotton and tomato) showed significant difference (Table 1). Population from Khandwa and Sriganganagar recorded the highest activity of glutathione-s-transferase with 1.71 folds (243.77± 6.56nmol/min/*B. tabaci* equivalent, 243.88± 25.66nmol/min/*B. tabaci* equivalent) for both the population on brinjal; 1.75 (263.53± 17.53nmol/min/*B. tabaci* equivalent) and 1.76 (265.74± 18.04nmol/min/*B. tabaci* equivalent) folds on cotton; 1.72 (289.28± 9.82nmol nmol/min/*B. tabaci* equivalent) and 1.66 (279.40± 23.21nmol/min/*B. tabaci* equivalent) folds on tomato as hosts respectively taking New Delhi population as unity. Populationfrom Ludhiana and Amravati recorded GST values of 1.29 and 1.12 folds on brinjal, 1.35 and 1.09 folds on cotton and 1.28 and 1.25 folds on tomato respectively. New Delhi population recorded the lowest value for GST among all the five populations evaluated with 142.65± 6.01nmol/min/*B. tabaci* equivalent on brinjal, 150.75± 7.71nmol/min/*B. tabaci* equivalenton cotton and 168.18± 7.88nmol/min/*B. tabaci* equivalent on tomato (Table 1).

Similar study was carried out by shifting the host from cotton to *Brassica* plants (white mustard and cabbage), resulting in increased expression of

Table 1. Activity of general esterase towards α–naphthyl acetateand GST activity towards CDNB in *B. tabaci*

Esterase towards α–naphthyl acetate

Population	Brinjal	RA	Cotton	RA	Tomato	RA
	Mean ± SE for rate of change of absorbance @450 (mOD/min/0.5 *B. tabaci* equivalent)		Mean ± SE for rate of change of absorbance @450 (mOD/min/0.5 *B. tabaci* equivalent)		Mean ± SE for rate of change of absorbance @450 (mOD/min/0.5 *B. tabaci* equivalent)	
New Delhi	1.96± 0.13^{a}	1	2.90± 0.23^{b}	1	3.17± 0.28^{b}	1
Ludhiana	2.68± 0.19^{a}	1.37	3.34± 0.2^{a}	1.15	5.02± 0.31^{b}	1.58
Sriganganagar	2.84± 0.17^{a}	1.45	3.27± 0.25^{a}	1.13	4.95± 0.2^{b}	1.56
Amravati	3.40± 0.65^{a}	1.74	4.67± 0.59^{ab}	1.61	5.19± 0.43^{b}	1.64
Khandwa	4.33± 0.48^{a}	2.21	4.83± 0.39^{a}	1.67	5.34± 0.38^{a}	1.69

GST activity towards CDNB

Population	Brinjal	RA	Cotton	RA	Tomato	RA
	Mean ± SE (nmol/min/ *B. tabaci* equivalent)		Mean ± SE (nmol/min/ *B. tabaci* equivalent)		Mean ± SE (nmol/min/ *B. tabaci* equivalent)	
New Delhi	142.65± 6.01^{a}	1	150.75± 7.71^{ab}	1	168.18± 7.88^{b}	1
Ludhiana	184.41± 11.1^{a}	1.29	204 ± 10.04^{a}	1.35	215.29± 13.54^{a}	1.28
Sriganganagar	243.88± 25.66^{a}	1.71	265.74± 18.04^{a}	1.76	279.40± 23.21^{a}	1.66
Amravati	159.20± 8.33^{a}	1.12	163.63± 7.6^{a}	1.09	209.67± 4.8^{b}	1.25
Khandwa	243.77± 6.56^{a}	1.71	263.53± 17.53^{ab}	1.75	289.28± 9.82^{b}	1.72

Values compared across host within population; Values followed by the same letters not significantly different at p= 0.10 after Tukey's HSD test; RA=Relative Activity

GST related genes by 1.97 to 2.08 folds. Whereas, corresponding decreased expression was observed switching back from white mustard to cotton. When a toxic glucosinolate of white mustard were added to the artificial diet a significant increase in expression was observed. Detoxification mechanism in insect is turned on and off depending on the levels of toxins in their environment (Alon et al., 2010).The GST values were higher in tomato as host but a significant difference across all the three hosts was reported only in populations like New Delhi and Khandwa. This is very well supported by work of Xu et al. (2014) who studied changes in activity of CarE, cytochrome P450 and GST in two biotypes of *B. tabaci*, 24 hrs post shift from cucumber onto various host plants. They found that the variations in cytochrome values were highest and that of GST value was lowest, as found in the present study where no significant variation across hosts was observed in population from Ludhiana and Sriganganagar.

Changes in activity of carboxyl esterases can be correlated with organophosphate resistance in certain insect species from Lepidoptera, Hemiptera and higher Diptera (Li et al., 2007).The involvement of elevated esterase activity in OP's and pyrethroid resistance had been clearly demonstrated in *B. tabaci* species complex (Alon et al., 2008; Byrne et al., 2000; Byrne and Devonshire, 1993; Dittrich et al., 1990; Hemingway and Ranson, 2000; Ishaaya et al., 1987 and Prabhaker et al., 1988). The enhanced GSTs expression has been implicated as resistance mechanism to DDT, OP's and pyrethroids in certain insects (Huang et al., 1998; Ranson and Hemingway, 2005 and Vontas et al., 2001). The higher values of esterases and GST in some populations may be due to high spray of conventional insecticides (organophosphates, carbamates and pyrathroids) before the use of newer molecules and led to development of high level of tolerance to conventional insecticides.

Thus, the present observations confirmed the induction effect of host plant on susceptibility changes of *B. tabaci* in response to several insecticides. Host plants can significantly influence the activity of several detoxifying enzymes in combination with insecticide selection pressure operating under different agroclimatic conditions. Esterase and GST recorded the similar enzyme pattern in host and population. Tomato recorded the highest value for both the enzymes followed by cotton and brinjal.The population from Khandwa recorded the highest values of esterase and GST indicating possible use of more insecticide molecules like organophosphates, carbamates and synthetic pyrethroids in this region compared to New Delhi which recorded the lowest value.

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