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Alkaline phosphatase activity in developmental stages of Asia I and Asia II-1 populations of whitefly, *Bemisia tabaci* (Gennadius)

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The whitefly, *Bemisia tabaci* (Aleyrodidae, Hemiptera), regarded as a species complex comprising over 34 genetic groups, is a serious insect pest of several crops. The aim of this study was to find out the difference in alkaline phosphatase (ALP) activity between developmental stages of two populations of *B. tabaci* belonging to Asia II-1 and Asia I genetic groups. There was a rise in ALP activity from first to second instar followed by gradual decline through third and fourth instars. ALP activity reached its peak during the adult stages of both Asia I and Asia II-1 populations. The specific activity of ALP in nymphal instars of Asia II-1 was significantly higher than that of Asia I.

The kinetics of ALP revealed that Asia I population showed significant increase in V_{max} value compared to Asia II-1 in the second instar, fourth instar and adult stages, whereas the opposite is seen in the first and third instars. Also, the Asia I population showed significant decrease in K_m value compared to Asia II-1 in the first and fourth instars; the opposite was seen in second instar. No significant differences were observed between the K_m values of the third instar and adult stages of both the populations. The possible physiological role of ALP and its implications in management of this pest are also discussed in this communication.

Keywords: Alkaline phosphatase, *Bemisia tabaci*, developmental stages, genetic groups.

WHITEFLY, *Bemisia tabaci* (Hemiptera: Aleyrodidae), one of the world's top 100 invasive pests with a host range of well over 900 plants (http://www.issg.org/database) causes serious damage directly as sap-sucking pest and indirectly as vector of plant pathogenic viruses. *B. tabaci* is regarded as a species complex comprising about 34 morphologically indistinguishable genetic groups[1]. There is a great amount of diversity of *B. tabaci* in Asia with the presence of 19 of the 34 genetic groups recorded so far. The Indian subcontinent represents an important Old World centre of diversification and evolution of *B. tabaci* with Asia 1 and Asia II-1 being the two dominant genetic groups widely distributed in India[2,3].

Alkaline phosphatase (ALP, EC 3.1.3.1; a ubiquitous hydrolase enzyme present in animals, fungi and bacteria)

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is responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins and alkaloids[4]. ALP has been documented in several species of insects, including *Leptinotarsa decemlineata*[5], *Bombyx mori*[6] and *Drosophila melanogaster*[7]. ALP activity was reported to be localized in several insect organs and tissues, including salivary glands, intestine and ovarioles in the B-biotype of *B. tabaci*[8]. ALP was reported to be involved in many key biological processes in insect, viz. development, nerve conduction, hormone synthesis, substance metabolism and caste differentiation[4]. Polyphagous insects were reported to be swift in adjusting their digestive enzymes for adapting to the changing nutrient conditions of their host plant(s) within a short time[9]. Comparative studies on ALP activities of two whitefly species on different host plants revealed that the quicker host plant adaptation of B-biotype of *B. tabaci* could be attributed to its ALP activity[10]. There is no information available on enzymatic attributes of two predominant *B. tabaci* genetic groups, Asia I and Asia II-1, prevalent in India. The present study was undertaken to find out differences, if any, in the kinetics of ALP activity in various developmental stages of these two genetic groups of *B. tabaci*.

The field populations of *B. tabaci* were collected during 2013 from New Delhi (28.6139°N, 77.2089°E) and Guntur, Andhra Pradesh (16.3008°N, 80.4428°E), India. The insects were collected from cotton plants by following standard sampling procedures. The puparia were sampled for valid species authentication. The respective populations were reared in insecticide-free exposure conditions on cotton (*Gossypium hirsutum*, L) at 27 ± 2°C, photoperiod of 14:10 h (L : D) and 60–70% of relative humidity in insect-proof climate-control chambers at the Division of Entomology, Indian Agricultural Research Institute, New Delhi, India.

The genetic group status of the two field populations of *B. tabaci* was ascertained using *mtCOI* primers. Genomic DNA was extracted from single *B. tabaci* adult using DNeasy blood and tissue kit (Qiagen) according to the manufacturer's protocol. The *mtCO1* region was amplified using forward primer CI–J–2195 (3′–TTGATTTTTTGGTCATCCAGAAGT–5′) in combination with reverse primer TL2–N–3014 (3′–TCCAATGCACTAATCTGCCATATTA–5′)[11]. The PCR mixtures consisted of 10 pmol of primer, 2.5 µl of 10× buffer, 2 mM $MgCl_2$, 2.0 mM dNTPs, 1U *Taq* DNA polymerase and the template DNA from individual insects from each population. The PCR cycling conditions were as follows: initial denaturation of 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 30 sec, annealing for 40 sec at 54°C, extension for 40 sec at 72°C followed by final extension at 72°C for 5 min in a thermal cycler (Biorad C1000 Thermal Cycler, USA). The amplified products were resolved by electrophoresis using 1% agarose gel with 1× TAE buffer in a submerged electrophoresis system at 70 V, then stained with ethidium bromide (1 µg/ml) and visualized in a gel documentation system (AlphaImager HP, Protein sample). Later, the PCR products were purified and sequenced. The mtCO1 sequences obtained from each population were subjected to homology search using Basic Local Alignment Search Tool (nBLAST) in NCBI (http://www.ncbi.nlm.nih.gov). Based on sequence analysis, the genetic group status of *B. tabaci* populations from New Delhi and Guntur was determined respectively, as Asia II-1 and Asia I (vide Gen Bank accession numbers of KF298440 for Asia II-1 and KF298441 for Asia I).

B. tabaci has six developmental stages, viz. egg, four nymphal instars and adult (Figure 1). Twenty individuals from each of the four nymphal instars and adult stages were homogenized in 100 µl of 10 mM sodium acetate–magnesium acetate solution (pH 7) at 4°C. Samples were centrifuged at 10,000 *g*, 4°C for 15 min, and the supernatants were collected for assaying enzymatic activity. ALP activity was estimated by incubating 20 µl of supernatant in 200 µl of substrate buffer containing 100 mM *N*-Tris-hydroxymethyl-1-methyl-3-aminopropanesulfonic acid (TAPS; pH 7.8) and 0.75 mM of *para*-nitrophenyl phosphate (*p*NPP) for 30 min. Then the reaction was stopped by keeping the plate in an ice bath for 5 min. Absorbance was measured at 405 nm in a Micro plate Reader (Molecular Devices, USA). *p*-Nitrophenol was also quantified at 405 nm to estimate *p*NPP hydrolysis. The enzyme unit (U/mg protein) is defined as the production of 1 nmol of *p*-nitrophenol by a reaction between 1 µg protein and related substrate (*p*NPP) per min at 37°C (ref. 10). Total protein content of the tissue samples was analysed using Bradford reagent with bovine serine albumin (BSA) as the standard. The enzymatic activity was calculated as *p*-nitrophenol produced (nmol/µl/min) and specific activity (nmol/µg/min) was determined as enzymatic activity (nmol/µl/min) divided by protein content (µg/µl).

V_{max}, the maximum initial velocity and K_m, the Michaelis constant were determined using specific activity of ALP at five different concentrations of the substrate for each developmental stage of the insect using the software program, Graphpad Prism® (version 6.05). Data were analysed by one-way ANOVA and multiple comparisons within population were made by Tukey–Honest Significant Difference (HSD). Mann–Whitney *U* test was performed for comparisons between data of two populations in SPSS program, as this test is considered to be more efficient for analysis of data for continuous measurements like enzymatic activity.

Table 1 presents the specific activities of the enzyme. Comparison of ALP activity between the two *B. tabaci* populations revealed that the specific activity in nymphal instars of Asia II-1 was significantly higher (about 1.2–1.5 times) than that of Asia I. The highest ALP activity was recorded in adult stages with values being 1.209 ± 0.093 nmol/µg/min and 1.225 ± 0.083 nmol/µg/min for Asia II-1 and Asia I respectively. However, the specific

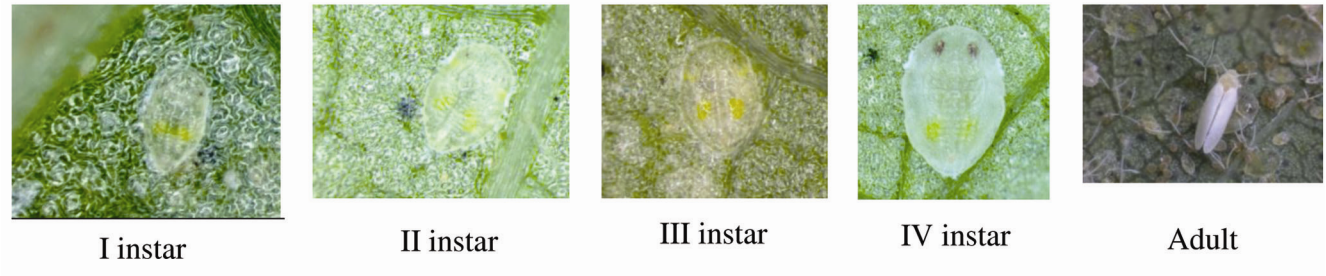

Figure 1. Nymphal and adult stages of whitefly, *Bemisia tabaci*.

Table 1. Specific activity of alkaline phosphatase (ALP) in developmental stages of *Bemisia tabaci* Asia II-1 and Asia I

Stage	ALP specific activity (nmol/μg/min)	
	Asia II-1	Asia I
First instar	0.671 ± 0.017^{aA}	0.478 ± 0.005^{aB}
Second instar	0.947 ± 0.007^{bA}	0.822 ± 0.005^{bB}
Third instar	0.866 ± 0.005^{bA}	0.720 ± 0.016^{bB}
Fourth instar	0.634 ± 0.004^{aA}	0.413 ± 0.004^{aB}
Adult	1.209 ± 0.044^{cA}	1.225 ± 0.047^{cA}

All values are mean of three replications. Numbers followed by same alphabet are not statistically different. Statistical significance within the population is indicated by small alphabets and between the populations by capital letters.

activities of the enzyme during the adult stages of both the populations were not statistically different (Table 1). There was a rise in ALP activity from the first to second instar followed by gradual decline through third and fourth instars. ALP activity reached its peak during the adult stages of both Asia I and Asia II-1 populations. The rise in ALP activity was seen in the active feeding stages of whitefly, i.e. early nymphal instars and adult stages. As the insects grow, they settle on the leaves remaining stationery (third and fourth instars). The fourth instar of whitefly is a quiescent stage (puparia) having no feeding activity and as such, ALP activity declined probably during the third and fourth instars.

Analysis of the kinetic parameters of ALP revealed significant difference in V_{max} and K_m values between the two whitefly populations. The Asia I population showed significant increase in V_{max} value compared to Asia II-1 in the second instar, fourth instar and adult stages, whereas the opposite was seen in the first and third instars (Figure 2). There was significant decrease in K_m value of Asia I as compared to Asia II-1 in the first and fourth instars, whereas the opposite was seen in the second instar. However, there was no significant difference between K_m values of the third instar and adult stages of both the populations. The adult stage showed lower K_m value compared to other stages for both the genetic groups under study (Figure 3).

The relationship between rate of reaction and concentration of substrate depends on the affinity of the enzyme to its substrate. This is usually expressed as K_m (Michaelis constant) of the enzyme, an inverse measure of affinity. If an enzyme has a small value of K_m, it achieves its maximum catalytic efficiency at low substrate concentrations. Considering the role of ALP in sucrose metabolism, the relatively high affinity of the enzyme in adult stages of Asia I and Asia II-1 populations (as indicated by significantly lower K_m values) indicates better host adaptation by these two cryptic species of *B. tabaci*.

Insect ALPs appear to have a pivotal role in absorption, cuticle formation and development[4]. Some studies have reported that mammalian and insect ALPs are involved in sucrose metabolism[12–14]. The whiteflies are piercing–sucking insects which feed on the plant phloem sap, a rich source of sucrose, and as such the insects require a sucrose concentration between 5% and 10% for maximum rate of metabolism while feeding[15]. Localization of ALP in salivary gland secretory system indicates their possible role in sucrose metabolism in *B. tabaci*[8].

The differential ALP activities recorded in Asia I and Asia II-1 populations of *B. tabaci* in this study are supported by similar studies showing significant differences in ALP activity in the developmental stages of two whitefly species, *B. tabaci* and *T. vaporariorum*[16]. While studying the effects of different host plants on ALP activity, it was observed that swift host adaptation of B-biotype of *B. tabaci* could be attributed to its better utilization of ALP for faster assimilation of sucrose[10].

B. tabaci has assumed the status of a global invasive pest with the rapid establishment of *B. tabaci* B- and Q-biotypes in over 35 countries in the past two decades. The diversity of *B. tabaci* in Asia is enormous, with the presence of 19 of its 34 genetic groups recorded so far[17]. With the distribution on large geographic range, higher genetic variability, capability to successfully colonize new areas and with the large host plant range, Asia I has emerged as the predominant cryptic species of *B. tabaci* in Asia. Recent survey of *B. tabaci* populations across the country has shown that Asia I is the most widely distributed *B. tabaci* genetic group in southern India, while Asia II-1 is the predominant species in northern India[17]. Several other investigations have elucidated the genetic variability (haplotypes diversity), wider distribution and broader

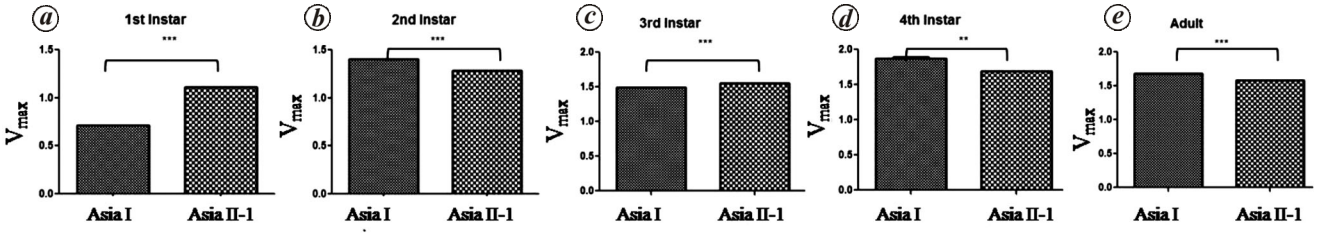

Figure 2 *a–e*. Comparison between V_{max} values of the Asia I and Asia II-1 populations at various growth stages. Asia I population shows significant increase in V_{max} value compared to Asia II-1 in the second instar, fourth instar and adult stages, whereas the opposite is seen in the first and third instars. $P < 0.05$(*), $P < 0.01$(**), $P < 0.001$(***), $P > 0.05$ (not significant).

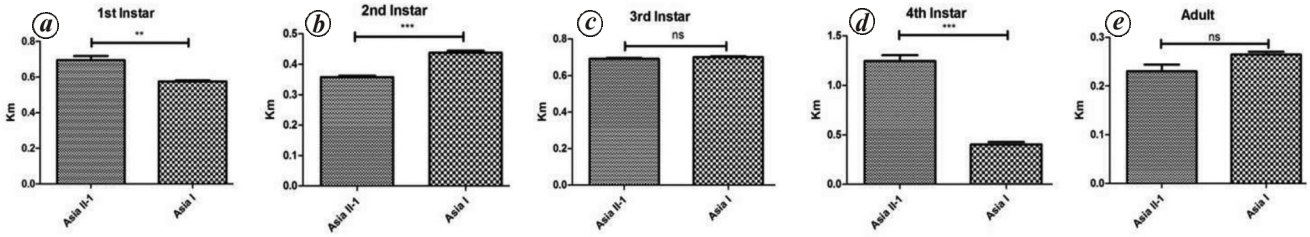

Figure 3 *a–e*. Comparison between K_m values of the Asia I and Asia II-1 populations at various growth stages. Significant decrease in K_m value of Asia I is seen compared to Asia II-1 in the first and fourth instars; the opposite is seen in the second instar. No significant difference is seen between K_m values of third instar and adult stages of both the populations. $P < 0.05$(*), $P < 0.01$(**), $P < 0.001$(***), $P > 0.05$ (not significant).

host range of Asia I cryptic species across the Asian countries[18–20]. The wider distribution and broader host range of Asia I may be attributed to its ALP activity.

Compared to that of B- and Q-biotypes, limited studies are available on Asian genetic groups of *B. tabaci*. The present study has thrown light on the ALP activities of the two predominant genetic groups of *B. tabaci* prevalent in India, viz. Asia I and Asia II-1. Further studies on ALP activity in Asia I and Asia II-1 vis-à-vis host plants and host shifts may elaborate on its role on host adaptation mechanisms. Understanding the biological traits of *B. tabaci* genetic groups would augur well for the sustainable management of this pest.

Ethics statement: No permits were required for collecting *B. tabaci* from the field, since it is not an endangered species affecting the biodiversity status.

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