IDENTIFICATION OF LARVAL MORPHOTYPES OF *Helicoverpa armigera* (Hübner) (LEPIDOPTERA: NOCTUIDAE) AND THEIR CHARACTERIZATION USING MOLECULAR MARKERS

By

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(2011-21-110)

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KERALA, INDIA

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DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR – 680656

KERALA, INDIA

2015

DECLARATION

I hereby declare that this thesis entitled **"Identification of larval morphotypes of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) and their characterization using molecular markers"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled **"Identification of larval morphotypes of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) and their characterization using molecular markers"** is a bonafide record of research work done independently by **Mr. Ranjith, M. T. (2011-21-110)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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Introduction

1. INTRODUCTION

Heliothine group of moths (Lepidoptera: Noctuidae) include some of the most damaging insect pests of agricultural crops throughout the world. *Helicoverpa armigera* (Hübner), *Helicoverpa assulta* (Guenée) and *Heliothis peltigera* (Denis & Schiffermüller) belonging to this group have been recorded from India (Jadhav and Armes, 1996). Erstwhile, *Helicoverpa rama* (Bhattacharjee and Gupta) has been distinguished as a species distinct from the commonly accepted *H. armigera* (Bhattacharjee and Gupta, 1972), but now it has been synonymyzed with *H. armigera.*

Helicoverpa armigera (Hübner), popularly known as American bollworm or gram caterpillar or pod borer or tomato fruit borer is the most dreaded one. It has been recorded on more than 181 plant species from 45 families in India (Manjunath *et al*., 1989) causing annual loss to the tune of ₹ 20,000 million (Ignacimuthu and Jayaraj, 2003). Combination of several factors like polyphagous nature, high mobility and fecundity, ability to undergo facultative diapauses (Fitt, 1989) and capacity to develop resistance against many synthetic insecticide groups (Armes *et al*., 1996; Ramasubramanian and Regupathy, 2004) have made it one of the world's worst pests (Pimbert *et al*., 1989).

In Kerala, the incidence of *H. armigera* was reported on tomato, okra, cowpea, bitter gourd and amaranthus (Mathew *et al.,* 1996; Levin, 2004; Levin *et al*., 2004). Subsequently, synergistic interaction of biocides and insecticides on tomato fruit borer was also studied (Levin, 2004) to evolve management strategies against the pest. However, the presence of larval morphotypes in different crop ecosystems is another feature of this pest. Differential susceptibility has also been reported in different colour morphs of *H. armigera* against various insecticides (Ghante *et al*., 2011), rendering management all the more difficult. This could be due to the presence of strong genetic variability with an adaptive significance for *H. armigera*.

Studies on phenotypic plasticity within (Fakrudin *et al*., 2007) and between (Patil *et al*., 2012; Basavanneppa and Balikali, 2014) populations of *H. armigera* collected from different host plants revealed that significant differences existed among the morphometric traits of *H. armigera* which displayed a structured pattern in the distribution of population.

Recent developments in molecular biology have resulted in several tools and techniques to analyze genomic variation at both individual and population level (Black *et al*., 2001). The DNA based marker system such as RFLP, RAPD, AFLP, SSRs, SNPs etc. have profound uses in area such as molecular ecology, molecular entomology, molecular systematics, population dynamics and diagnostics (Morin *et al*., 2004).

Genetic variability studies of *H. armigera* on different crops in India were carried out using RAPD (Fakruddin *et al*., 2004; Deepa and Srivastava, 2011; Yenagi *et al*., 2012) and SSR markers (Subramanian and Mohankumar, 2006). Among the molecular markers, SSRs are highly informative, with high degree of polymorphism, co-dominant nature, and coverage of multiple loci make them better in measuring the genetic structure of *H. armigera* populations (Scott *et al.*, 2003) than RAPD markers. Moreover, the DNA fingerprint profile based on SSR markers could be used to evaluate DNA variability at individual and population level (Hoy, 2013).

Thus phenotypic and genetic variability studies could yield valuable information on population structure that will be useful in evolving strategies for management of genetically distinct morphotypes in the larval stages itself.

Hence, the present investigation on "Identification of larval morphotypes of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) and their characterization using molecular markers" was undertaken with the following objectives:

1. Identification of *H. armigera* morphotypes by comparing different phenotypic traits.

2. Apportioning of larval morphotypes into morphoclusters based on the phenotypic traits.

3. Evaluation of genetic variability of *H. armigera* larval morphotypes using molecular markers.

Review of Literature

2. REVIEW OF LITERATURE

Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae) is a polyphagous pest occurring on cotton, tomato, okra, chickpea, pigeonpea, chilli and many other crops, inflicting substantial crop losses every year (Reed and Pawar, 1982; Manjunath *et al*., 1989; Sharma, 2001). The existence of different larval colourmorphs of *H. armigera* and the dominance of specific colourmorphs across different host plants were documented (Patil, 2005; Basavanneppa and Balikali, 20I4). It is envisaged in the present study to identify the larval morphotypes of *Helicoverpa armigera* (Hübner) and their characterization using molecular markers. The literature pertaining to the objectives enlisted in the introductory chapter is presented hereunder.

2.1. Morphological characterization of *Helicoverpa armigera*

The ability of *H. armigera* to thrive on diverse host plants is characterized by its high mobility, fecundity and ability to develop resistance against synthetic insecticides used in its management (Armes *et al*., 1996; Kranthi, 1997; Ramasubramanian and Regupathy, 2004). Though differences exist in the behaviour of *H. armigera* populations infesting various host plants and geographically distinct groups, the existence of sub specific differences between populations cannot be completely ruled out (Reed and Pawar, 1982). There have been consistent variations in the morphological parameters that merited specific separation within (Bhatnagar and Davies, 1978) and between populations of *H. armigera* collected from different hosts (Bhattacharjee and Gupta, 1972).

2.1.1. Larval characters

The neonates (freshly emerged larvae) of *H. armigera* were translucent yellowish white and 1.0 to 1.5 mm length with the head, prothoracic shield, supra anal shield and prothoracic legs being dark brown to black in colour. The larvae have a spotted appearance due to sclerotized setae, tubercle bases and spiracles (King,

1994; Bhatt and Patel, 2001). The second instars were yellowish green with black thorasic legs. The number of larval instars of *H. armigera* varied between five and seven, however commonly reported was six (Nadgauda and Pitre, 1983). The full grown larvae were brown or pale green with brown lateral stripes and a distinct dorsal stripe. Larvae were long and ventrally flattened but convex dorsally. Larval size in final instars ranged from 3.5 to 4.2 cm (King, 1994).

2.1.1.1. Colour polymorphism

Colour polymorphism has been defined as the presence of two or more distinct, genetically determined colourmorphs within a single interbreeding population (Gray and McKinnon, 2006). Though the basic mechanism maintaining most polymorphism in nature are unknown, Losey *et al*. (1997) derived a mathematical model that determined the coexistence of both the red and green colour morphs of pea aphid, *Acirthosiphon pisum* (Harris) in a population as controlled by density dependent parasitism and or predation.

The larval colour in *H. armigera* was quiet variable, the young caterpillars were pale green, but the colour of later instars varied from yellowish green to dark brown and with different markings (Rao and Abraham, 1956; Gopalan and Venugopal, 1972).

Srivastava and Bhatnagar (1964) reported that *H. armigera* caterpillars were yellowish on hatching, whereas according to Singh and Singh (1975) the newly hatched larvae were translucent and yellowish white with faint yellowish orange longitudinal lines. The head, the thoracic shield, the anal shield and legs were brown and setae were dark brown.

Full grown caterpillar was greenish with dark brown lines along the sides of the body (Gopalan and Venugopal, 1972), green to light brown with longitudinal grey streaks along the sides (Lewin *et al*., 1973). The colour variation in the larvae ranged

from green, fawn, pink, yellow or brown and very dark to light green or pink (Uthamasamy *et al*., 1988).

According to Ramos and Rejesus (1976), *H. armigera* showed nearly the same pale brown colouration during early stages, but different body colour became apparent during the mid instars and was pronounced in the final larval instars.

Larval colour in *H. armigera* was affected by diet (Ramos and Rejesus, 1976). The larvae were fed with five different plants (i.e. corn ear, cotton bolls and leaves, tomato leaves and fruits, tobacco leaves and artificial diet using mungbean seeds) and investigated their body colour. They obtained both green and brown larvae fed on four plants except for tobacco leaves on which all larvae showed green body colour. It was also revealed that the addition of ß-carotene in the artificial diet intensified the dorsal stigmata colouration and caused early appearance of various larval colour forms.

Rajagopal and Channabasavanna (1982) observed the instar wise colouration in *H. armigera* as yellow tinge of green in first instar, greyish yellow in second instar, greyish yellow with light brown during third instar, light yellowish green to dark brown during fourth instar, pale greenish to yellowish brown during fifth instar and yellowish white or greenish yellow or greenish brown to dark brown during sixth instar.

Larval colouration was closely associated with nutrition and they exhibited different colours and markings when reared on cotton, corn, tomato and tobacco (Jyothi, 1991). *H. armigera* larvae were yellowish white to reddish brown in the early instars, whereas in the later instars colour was extremely variable ranging from shades of green, straw yellow, black, pink or reddish brown (EPPO, 2003).

Occurrence of nine different colour morphs of *H. armigera viz*., greenish brown, dark green or black green, greenish, whitish, light green, reddish brown,

yellowish brown, blackish brown and reddish tinge with green have been documented in south Indian cotton ecosystem (Vijayakumar, 2005).

Yamasaki *et al*. (2009) studied the effect of host plant parts of tomato, cotton, okra, spider flower and cosmos on larval body colour polymorphism in *H. armigera* and observed that the frequency of green colouration in final instar larvae reared on leaves were significantly higher than that of larvae reared on fruits and or flowers. Larvae also had a certain degree of plastic response to the diet change, which indicated that larvae were able to adjust body colour according to the host plants on which they fed.

Though genetic control of colouration in *H. armigera* was not known, variability within basic colouration was obvious indicating that a complex set of modifiers must be in operation and that change in quantitative fashion (Jameson and Pequegnat, 1971). Bartlet and Raulston (1982) reported that the colour character was under genetic control, whereas Maragal (1990) observed that the green coloured morph readily acquire yellow colour of petals on feeding, while black colour morphs retained melanin to some extent. Furthermore, Mo *et al*. (2005) observed black phenotype mutant strains of adults and pupae in *H. armigera* were pronounced by one recessive gene.

2.1.1.2. Larval length, width and weight

The full grown larva of *H. armigera* measured about 35-42 mm in length (Uthamasamy *et al*., 1988). Tripathy and Singh (1999) reported that the full grown larvae collected from well matured chickpea plants during the month of April possessed the maximum body length (34.22 mm), width (6.50 mm) and weight (438.31 mg).

Fakrudin *et al*. (2007) found difference in larval length and weight among populations of *H. armigera* in South Indian cotton ecosystems. The maximum larval

weight was recorded in Guntur populations (505.75 mg) which was on par with Raichur populations (501.40 mg), whereas, the lowest larval weight was recorded in Madurai populations (480.07 mg). Raichur population recorded the maximum larval length (27.08 mm) whereas larval population from Madurai recorded the lowest larval length of 22.42 mm.

Ali *et al*. (2009) studied the morphometrics of various life stages of *H. armigera* on chickpea and reported that the fifth instar recorded average larval length and width of 20.97±0.61 mm and 3.25±0.61 mm respectively.

The investigation on biometrics of *H. armigera* on pods of the three gram varieties revealed that the larvae (fifth instar) reared on variety 'Virat' recorded the highest body length (19.67±0.58 mm), body width (2.85±0.05 mm) and weight (162.47±2.46 mg (Dhurgude *et al*., 2009).

Sharma *et al*. (2011) recorded the measurement of different stages of tomato fruit borer, *H. armigera* and among these different stages fifth instar larva recorded the height length of 32.40±2.83 mm and width of 5.20±0.02 mm.

Phenotypic plasticity in *H. armigera* population occurring in nine host plants were explored and it was observed that larval weight and length varied significantly across the populations, though larval width did not vary significantly. *H. armigera* on okra recorded a lower larval length, width and weight of 30.45±4.0 mm, 2.64±0.36 mm and 295.30±16.9 mm respectively, whereas *H. armigera* on chickpea recorded the highest larval length (31.44±3.73 mm), width (2.98±2.26) and weight (305.80±52.8 mm) (Patil *et al*., 2012).

Basavannappa and Balikali (2014) studied the morphological variability of *H. armigera* on ten host plants. Fifth instar larvae collected from chickpea recorded a larval length, width and weight of 29.85±3.49 mm, 1.97±0.51 mm and 323.3±53.82 mg respectively. While the corresponding figure for larvae on okra were 28.40±4.34

mm, 2.37±0.43 mm and 284.65 mg respectively. Length, width and weight recorded were 25.75±3.57 mm, 2.17±0.40 mm and 271.10±20.18 mg respectively in case of larvae reared on tomato.

Gadhiya *et al*. (2014) studied the morphometry of *H. armigera* on groundnut leaves under laboratory conditions. Fifth instar larvae recorded a length of 28.76±1.05 mm and breadth of 3.68±0.33 mm. Morphometry of different instars of *H. armigera* reared on chickpea was explored and the fifth instar recorded larval length and width of 20.97±0.60 mm and 3.25 ±0.04 mm, respectively (Singh *et al*., 2014).

2.1.1.3. Larval chaetotaxy

Chaetotaxy refers to the presence or absence or relative lengths and positions of body setae. Formal keys for the identification of caterpillars were highly depending on chaetotaxy, particularly the primary setae, those that are broadly homologous across lepidopteran families. A few lineages are also possessing additional secondary setae. The basic number of primary setae on body segment is eleven and variation in their number and position occur among species, genera and families, accordingly setal arrangement are often used in taxonomic identity.

Amate *et al*. (1998) developed taxonomic keys to distinguish *H. armigera* from related species by the prothoracic setal characters. Prothorax of *H. armigera* possess two sub dorsal setae and two lateral setae, lateral setae on prothorax horizontally aligned with spiracles. According to Gilligan and Passoa (2004) the dorsal setae, D of abdominal segment one to eight (A_{1-8}) inserted on large conical chalaza, those of A_1, A_2 and A_8 were often larger than the rest. The larval body colour of *H. armigera* was highly variable, but usually with lines, stripes and sometimes a black bar joining the D setae of A_1 or A_2.

2.1.2. Pupal characters

2.1.2.1. Pupal length, width and weight

Pupa of *H. armigera* was dark tan to brown in colour, 14-22 mm long and 4.5-6.5 mm wide. Body was rounded both anteriorly and posteriorly with two long tapering parallel spines at posterior tip (Sullivan and Molet, 2007).

According to Ali *et al*. (2009), pupa of *H. armigera* was obtect type with mahogany brown in colour. The surface was smooth and rounded both interiorly and posterior with two tapering parallel spines at tip. The average length and width of pupa was 19.00±0.30 mm and 5.72±0.08 mm respectively

Helicoverpa armigera pupa from chickpea plants was found to possess the highest length (20.01 mm), width (6.51 mm) and weight (391.19 mg) (Tripathy and Singh, 1999). According to Brochet *et al*. (2003) the pupa of *H. armigera* ranged from 14 to 22 mm long and 4.5 to 6.5 mm in width at the widest point and dark brown colour with a smooth surface.

Fakrudin *et al.* (2007) studied morphometric variation in geographic population of cotton bollworm *H. armigera* occurring in South Indian cotton ecosystem. They observed a highly significant difference in pupal length (14.63 mm to 19.17 mm) and pupal weight (244.14 mg to 276.30 mg) among the population collected from different locations.

Patil *et al*. (2012) investigated the morphometric plasticity for selected traits in larvae and pupae of *H. armigera* occurring on different host plants, among them pupae collected from chickpea recorded the highest length and weight of 16-22 mm and 167-311 mg respectively, whereas pupae on okra recorded a lower length of 13-21 mm and weight of 161-281 mg.

Gadhiya *et al*. (2014) recorded the average length and breadth of male pupa as 21.09±1.12 mm and 5.05±0.46 mm respectively, whereas in female the average pupal length and breadth recorded was 21.37±1.74 mm and 5.80±0.49 mm respectively.

Studies on morphometrics of gram pod borer *H. armigera* reared on chickpea recorded an average pupal length and breadth of 19.00±0.30 mm and 5.72±0.08 mm respectively (Singh *et al*., 2014).

2.1.3. Adult characters

2.1.3.1. Length and width of fore wing, length of fore, mid and hind femur

The adult moth of *H. armigera* is stout bodied with a typical noctuid moth appearance having wing span of 35-40 mm and body length of 18-19 mm. The colour varied from dull greenish yellow to olive grey or light brown and females were darker than males (King, 1994). Brambila (2009) reported that the male moths of *H. armigera* were usually yellowish brown, light yellow or light brown and female moths were orange brown in colour. Forewing have a black or dark brown kidney shaped marking near the centre and hind wings are creamy white with a dark brown or dark grey band on the outer margin.

Adult moths of *H. armigera* have two distinct forms; one with grey forewing while the other with yellowish brown. The dark spot on the forewing wing often form a 'V' shaped mark. The hind wings were dull grey with a dark edge. The wing span was upto 40 mm and the female moths were larger than males (Nylin and Gotthard, 1998).

The maximum adult length at full wing expanse recorded from the *H. armigera* population collected during March (35.79 mm), which was very closely related with that of population collected and observed during February (35.78 mm) and April (35.04 mm), whereas the difference in morphological parameters was not

much pronounced in population collected during the rest of the months (Tripathy and Singh, 1999).

Fakrudin *et al*. (2007) recorded the morphological variability in geographical populations of *H. armigera* from South Indian cotton ecosystems. Significant difference in length of forewing among the population was recorded. The length of forewing ranged from 13.70 to 17.09 mm across the population collected from different locations, whereas width of forewing ranged between 6.58 mm and 7.44 mm. Similarly, a highly significant difference was recorded for length of fore, mid and hind femur.

Among the morphometry of different stages of *H. armigera* studied, the male moth recorded a length and breadth (wing expanded) of 17.55±0.52 mm and 34.62±1.49 mm respectively, whereas in female moth, the length and breadth (wing expanded) of 21.09 ±1.28 mm and 40.77±1.68 were recorded (Gadhiya *et al*., 2014).

2.1.3.2. Adult male and female genitalia

As in any other taxonomic study, identification of species by means of genitalia depends upon a valid recognition of morphological characters (Siverly, 1947). Many authors have recognised the importance of genitalia as the most informative specific character in Lepidoptera (Niculescu, 1976), especially due to their variation in the valves of male genitalia (Fox, 1953).

According to Hardwick (1965), the male genitalia of *H. armigera* possessed vesica with a prominent spine in the basal region, clusters of spines on the coils of vesica, basal pouch of vesica without a slender posterior diverticulum, a broad anterior diverticulum and two small dorsal diverticula, the vesica terminating apically in a normal coil and valve of the genitalia five times as long as wide. In female genitalia, dorsal sclerotisation at base of appendix bursae was restricted, the appendix

bursae terminating apically in a normal dilation and lumen surface of appendix bursae clothed with spicules.

The male genitalia of *Helicoverpa* spp. possess valves which were long and thin, without projection and a row of inward-curved spines at the apical margin. The number of cornuti inside the aedeagus varied between *H. armigera* and *H. zea.* If the numbers of cornuti sets are 12 or less it could be *H. armigera*. If the count of cornuti exceeds 12, it is considered to be *H. zea* (Brambila, 2009).

Length of genital capsule, valves, ejaculatory duct in male and ductus bursae and appendix bursae in female were observed for possible variation *in H. armigera* occurring on different host plants. The *H. armigera* male population from chickpea recorded significantly higher length of genital capsule, valves and ejaculatory duct. With respect to length of ductus bursae and appendix bursae in female, significantly higher length was recorded in *H. armigera* population collected from chickpea (Patil *et al*., 2012).

2.2. Molecular characterisation of *Helicoverpa armigera*

Knowledge on genetic makeup and population structuring of an insect such as *H. armigera* with its diverse habitat would help greatly in the development of control methods whether cultural, biological or chemical. The population may be structured in other ways such as development of certain morphs or haplotypes which prefer to feed on various host plants may eventually leads to speciation. Thus it is important to determine genetic variation in a population and study their pattern of distribution.

Over a long time significant contribution have been made in the field of systematics through morphometrics wherein a number of difficulties were encountered due to genotype-environment interaction. The limitation in using morphological, physiological and cytological markers for assessing genetic diversity and population dynamics have been largely circumvented by the developments in

DNA based markers (Cruickshank, 2002). The advances in molecular biology enabled the researchers in entomology to understand the genotype of an individual and to estimate the genetic relatedness and genetic distances between two or more individuals.

Molecular markers in nature are neutral to the stages of development, physiological status and environmental influences (Black *et al*., 2001; Haeckel, 2003). Isozyme electrophoresis was the first technique to become widely available as tool in taxonomic and population studies. The Isozymes and other protein markers are often expressed codominantly and discriminate homozygous and heterozygous individuals. However, the limited number of proteins and isozymes as markers and requirement of different protocols for each enzyme/protein limit their utility. Unlike morphological and protein-based markers, several DNA based markers are available to elicit the differences between individuals and populations, or they can be developed for each specific purpose.

The DNA based marker system extensively used in entomological investigation of *H. armigera* includes restriction fragment length polymorphisms (RFLPs), randomly amplified polymorphic DNAs (RAPDs), mitochondrial DNA (mtDNA) markers**,** microsatellites/simple sequence repeats (SSRs) (Fakruddin *et al*., 2006).

Restriction Fragment Length Polymorphism

Restriction Fragment Length Polymorphism (RFLP) is a class of polymorphism that arises due to difference in nucleotide base sequence at positions called restriction sites of specific restriction endonucleases (RE) on the DNA resulting in varied size of DNA fragments. The alternative RFLP phenotypes at a given locus are determined as length polymorphisms, following electrophoresis of genomic DNA digested with one or more REs.

To discriminate *Helicoverpa armigera* from *Helicoverpa assulta,* a 557 base pair position of 16S Ribosomal RNA gene of mitochondrial DNA was analysed by PCR-RFLP. Results of nucleotide sequence of amplified DNA product from two species showed that they could be easily discriminate from each other by 'Msel' or 'Vspl' digestion of amplified product (Orui *et al*., 2000).

Kranthi *et al*. (2005) used PCR-RFLP tool to differentiate between *H. armigera* and *H. assulta* infesting various crops in India. The mid cytochrome region with high functional significance was chosen for study. The amplified product digested with 'RasI' yielded two fragments in *H. armigera*. This fragments were absent in *H. assulta* due to the absence of restriction digestion.

Random Amplified Polymorphic DNA markers

This is a PCR based marker system where genomic segments are amplified using oligo nucleotide primers. Generally random decamer primers are used to prime the synthesis of DNA from homologous sites on the test DNA in PCR. A diverse array of molecular technique is available for high resolution of genetic studies of population level processes. Among them, Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) using a single primer amplifies many regions of genomic DNA (Williams *et al*., 1990).

Zhou *et al*. (2000) analysed the genetic structure of cotton bollworm, *H. armigera* in Eastern Mediterranean using RAPD-PCR. Moths were sampled in five locations in Israel and one location in Turkey. The result revealed that a low level of genetic distances existed between Israeli and Turkish population.

Study on genetic variability of cotton bollworm, *H. armigera* of South Indian cotton ecosystems was carried out using RAPD markers, which revealed that the geographic population showed a similarity less than 42 per cent indicating a high level of genetic difference between populations (Fakruddin *et al*., 2004).

Deepa and Srivastava (2011) explored the genetic diversity of *H. armigera* populations collected from different agroclimatic zones of India by RAPD markers. Molecular data analysis clustered the *H. armigera* population into two groups. The first group (X) composed of eight populations which was further subdivided into X1 and X2 at similarity coefficient of 0.13, whereas the distinct group (Y) consisted of only Dharwad population.

Investigation on molecular diversity of *H. armigera* from five different regions of Northern Karnataka revealed that the genetic distance of 25.8 per cent was observed between Raichur and Bijapur population. The least genetic distance of zero per cent was found between Dharwad and Haveri population, Dharwad and Belgaum population and Haveri and Belgaum population (Yenagi *et al*., 2012).

Rahman *et al*. (2014) analysed genetic variability of *Helicoverpa armigera* at different ecological zones of Bangladesh in comparison with Indian population. It was observed that *H. armigera* population from Bangladesh had 25-45 per cent similarity and in Indian population similarity remained within this range.

Mitochondrial DNA (mtDNA) markers

Mitochondrial genome is maternally inherited. Any changes in the mitochondrial DNA (mtDNA) are transmitted to the entire progeny. Evolutionary changes in the conserved regions of mtDNA spread rapidly within population. The Cytochrome Oxidase-I (CO-I) region of mt DNA is the most studied region of the insect mitochondrial genome. Analysis of this mtDNA provides insight to understand the natural genetic diversity and population structures in organisms (Avise, 1994).

Mitochondrial DNA analysis were carried out for field populations of *H. armigera* sampled from Australia, Burkina Faso, Uganda, China, India and Pakistan which were associated with various host plants. Single nucleotide polymorphisms (SNPs) with the partial COI gene differentiated *H. armigera* populations into 33

mtDNA haplotypes. Phylogenetic analysis of four major *Helicoverpa* pest species indicated that *H. punctigera* is basal to *H. assulta*, which in turn is basal to *H. armigera* and *H. zea* (Behere *et al*., 2007b).

Genetic diversity analysis of twelve cotton bollworm, *H. armigera* populations from different geographic regions of South India was done using mitochondrial DNA-specific markers. The population showed a varied degree of genetic similarity within a range of 0.04-0.52. Also, the populations appeared to be more dispersed on the principal component plot indicating a wide genetic base. On a larger scale, genetic differences among the populations appeared to have resulted from low dispersal rates between populations (Vijaykumar *et al*., 2008).

Genetic diversity of tomato fruit borer, *H. armigera* inferred from mitochondrial cytochrome oxidase-I (mtCO-I) analysis showed that there was no significant variations in the CO-I sequences of *H. armigera* collected on various hosts and geographical locations. However, the phylogenetic tree indicated the possibility of emerging host associated genetic differences in *H. armigera* populations (Asokan *et al*., 2012a).

Molecular diversity of genes, namely actin, glutathione-S-transferase (gst), cytochrome P450, chymotrypsin and serine protease from the intraspecific populations of the fruit borer *H. armigera* was studied to understand the sequence polymorphism. Phylogenetic analysis concluded that these intraspecific populations from India formed a major clade. Comparison of deduced amino acid sequences concluded that there were no major differences except for serine protease. This analysis would be useful for delineating genetic relationships amongst the intraspecific populations and estimating genetic diversity, thereby gaining insight into genetic structure of populations (Asokan *et al.*, 2012b).

Simple Sequence Repeats (SSRs) or Microsatellite markers

Simple Sequence Repeats (also termed as micro satellites) are stretches of DNA consisting of tandemly repeating by mono-, di-, tri- or penta-nucleotide units that are arranged throughout the genome of most eukaryotic species. The variation in SSR loci is due to the differences in number of repeats, which primarily arises as errors during DNA replication. Using specific forward and reverse primers designed for sequences homologous to the flanking sequences of the repeat unit and length variation in repeat can be detected by PCR (Fakruddin *et al*., 2006)

Microsatellite markers are highly reproducible, and amenable for multiplexing, hence throughput approach is possible. They are codominant (except for null allele) and can detect variation within and between insect populations. DNA finger printing using microsatellite can be done with the PCR using specific or consensus primers (Kirby, 1990).

Often the SSR amplicons are separated on poly acrylamide gels to detect polymorphism that could be due to a few base differences. Silver staining procedure is generally adopted to detect them on acrylamide gels whenever ethidium bromide stained agarose gels do not resolve the amplicons (Fakruddin *et al*., 2006).

The primary application of SSRs has been in genetic diversity and genetic linkage map construction. SSR markers have been successfully used in paternity studies of Hymenopteran insects (Estoup *et al*., 1995) and genetic fluxing of lepidopteran insects (Ananthakrishnanan, 2005). In aphids, hymenopteran insects, mosquitoes, moths and butterflies, SSR markers have provided useful information on genetics of population (Black *et al*., 2001). Lehmann *et al*. (1997) used these markers in mosquitoes for genetic studies at population level.

The characteristics of SSR markers such as coverage of multiple loci, co-dominanace and high polymorphism suit them better in task of measuring genetic structure in *H. armigera* (Scott *et al*., 2004). The use of SSR markers for *H. armigera*

was previously hampered by non availability of DNA sequence information. Recently many SSR markers specific for *H. armigera* have been identified (Tan *et al*., 2001; Scott *et al*., 2004; Ji *et al*., 2003).

Grasela and McIntosh (2005) tested the primers previously designed to amplify microsatellite DNA markers in the Old World bollworm, *H. armigera* in three closely related species. The corn earworm, *Helicoverpa zea*; tobacco bud worm, *Heliothis virescens* and *Heliothis subflexa*. Of the fourteen loci surveyed, only four loci HaB60, Hac14, HaC87 and HaSSR1 considerably demonstrated scorable single copy of microsatellite bands. Of these four, length polymorphism was identified only in the HaB60 marker (160bp, 140bp) of the *Heliothis virescens* and *Heliothis subflexa* sampled laboratory population.

A novel set of five polymorphic di-or tri-nucleotide microsatellite loci suitable for population genetic study were developed from an enriched genomic library for the cotton bollworm, *H. armigera* and cross amplifiability of these and other published loci was tested in a closely related species *H. assulta*. The expected heterozygosity at these loci ranged from 0.62 to 0.91 in the cotton bollworms and the observed allele numbers varied from 4 to 12 in the limited number of individuals tested (Ji *et al*., 2005).

Subramanian and Mohankumar (2006) studied the genetic variability of *H. armigera* occurring on different host plants using microsatellite simple sequence repeat (SSR) markers and found that the *H. armigera* population on tomato and okra were found to be closely related whereas population on cotton and black gram differ widely. The population on cotton was found to be only distantly related to all other hosts.

Endersby *et al*. (2007) characterized population differentiation in Victorian samples of *H. armigera* using eight microsatellite loci and found no evidence of genetic structure among samples collected at different times. Moreover, Victorian

samples were not differentiated from other samples of *H. armigera* from Queensland and New Zealand. All the samples showed substantial deviations from Hardy-Weinberg equilibrium, suggesting a high frequency of null alleles typically found in microsatellites of Lepidoptera. These results indicated that populations of *H. armigera* were not strongly structured among regions in South-Eastern Australia.

The genetic structure of *H. armigera* population across sub-Saharan cotton belt was studied using ten polymorphic microsatellite markers. Despite the high polymorphism (5-50 alleles/locus) the result revealed a low level of genetic distances among locations, collection dates and host plants (Vassal *et al*., 2008).

Khaiban *et al*. (2010) studied the genetic variability of geographical population of the bollworm *H. armigera* in West and North West Iran using ten different SSR primers. The highest number of fourteen markers were produced by the primer HaSSR1, followed by nine markers by HaSSR6, with high degree of polymorphism of 75- 100 per cent. The primers HaSSR4, HaSSR6, Hac87, and HaD47 were found to be highly informative to differentiate the population with polymorphism information content value of 100 per cent. Cluster analysis based on molecular data assigned the studied pod borer moth populations into two groups.

Chen *et al*. (2011) reported that *H. armigera* specific nuclear DNA marker can be employed to reliably discriminate between *H. armigera* and *H. assulta* by simple polymerase chain reaction amplification. On testing with HaSSR1, one diagnostic band of about 130 bp was specifically amplified in *H. armigera*, while in *H. assulta* and *H. punctigera*, either several non specific bands were produced or no band was amplified.

Microsatellites can be generated by analysis of sequences available in GeneBank, especially for species that have had their genome sequenced. Alternatively, microsatellite-enriched library can be generated, although this can be time consuming, costly and technically complex. These markers are useful to monitor

gene flow, discrimination of parent-offspring, forensic and genome divergence studies, construction of physical maps (Hoy, 2013).

Gut metagenomics of *H. armigera*

Helicoverpa armigera (Hübner), harbour diverse gut bacterial communities and it helps in modifying their feeding behaviour in different crop ecosystem. The presence of beneficial microorganism in the gut (gut microbiota) play a major role in upgrading the nutrient status of diet, aids in digestion of recalcitrant food, protection from parasites, pathogens and development and maintenance of host immune system (Gill *et al*., 2004; Wernegreen, 2002).

Gut microbiota of insects are composed of a wide variety of species, including bacteria, archaea, and eukaryea. Previously, the gut bacterial community of termite (Warnecke *et al*., 2007) have been studied in detail, similarly total bacterial community from intestinal tract of fruit fly, *Bactrocera minax* Enderlein, Diptera (Wang *et al*., 2014) and red palm weevil, *Rhynchophorus ferrugineus* (Olivier), Coleoptera (Montagna *et al*., 2015) have also been reported.

The gut bacteria community of *H. armigera* was previously analysed by isolation and cultivation techniques (Madusudan *et al*., 2011) and polymerase chain reaction (PCR) based cloning methods (Priya *et al*., 2012) resulted in identification of few groups of bacteria.

16S ribosomal RNA (rRNA) gene sequencing was the most popular method adopted earlier to identify bacteria (Petti *et al*., 2005). But, it cannot be employed to reveal polymicrobial specimen wherein multiple templates resulted uninterpretable Sanger reads (Drancourt, 2000). However, the development of high throughput next generation sequencing with the primer spanning hypervariable regions (V1-V9) of 16S rRNA gene circumvent the limitations of earlier used

methods and enlighten the way to identify total bacterial community and their subsequent classification.

Materials and methods

3. MATERIALS AND METHODS

The details of materials used and the methodology adopted during the course of the investigation on "Identification of larval morphotypes of *Helicoverpa armigera* (Hübner) and their characterization using molecular markers" conducted at Department of Agricultural Entomology, College of Horticulture, Kerala Agricultural University, Vellanikkara are as follows.

3.1. Morphological characterisation of *Helicoverpa armigera* (Hübner)

3.1.1. Sampling of *Helicoverpa armigera* population

Surveys were conducted in vegetable growing fields of Palakkad, Thrissur, Kasaragod and Thiruvanathapuram districts of Kerala and flower growing fields of Thovala of Tamil Nadu (Table 1) during the period from 33rd standard week of 2012 to 5th standard week of 2014. Since more incidence of *H. armigera* was observed in Thrissur and Palakkad, a purposive sampling was carried out in tomato fields of Palakkad as well as tomato, okra, chickpea and amaranthus fields of Thrissur to collect different larval morphotypes of *H. armigera*. From one acre of crop field fifty larve of *H. armigera* were collected and brought to laboratory with plant parts in plastic boxes containing twelve cavities with each cavity measuring 6.5 cm $\times$ 6.0 cm $\times$3.5 cm.

3.1.2. Rearing of *Helicoverpa armigera* larvae in the laboratory

Helicoverpa armigera larvae collected from tomato, okra, chickpea and amaranthus were brought to laboratory and reared on the respective plant parts on which they were feeding. The larvae collected from tomato were also fed with semi synthetic diet to study the difference in their morphometric parameters.

Table 1. Details of surveys conducted during the period of study

Sl. No	Location	Crop	Period of collection			Interval of collection
			2012	2013	2014	
1	Vellanikkara, Thrissur	Tomato	41st to 44th SW	40th to 44th SW	NA	Weekly
		Okra	44thto 47th SW	7th to 10th SW	4th to 5th SW	Weekly
		Chickpea	49th to 52nd SW	1st SW	NA	Weekly
		Amaranthus	NA	19th to 22nd SW	NA	Weekly
2	Kozhinjambara, Palakkad	Tomato	33rd to 41st SW	35th to 41st SW	NA	Fortnightly
3	Thaikkadappuram, Kasaragod	Okra	NA	20th SW	NA	--
4	Vellayani, Thiruvananthapuram	Okra	40th SW	45th SW	NA	--
5	Thovalai, Kanyakumari	Marigold	40th SW	NA	NA	--

SW-standard week NA-not available

3.1.2.1. Preparation of semi synthetic diet of *Helicoverpa armigera*

Helicoverpa armigera larvae collected from tomato fruits were reared in the laboratory using semi synthetic diet (Armes *et al*., 1992) (Table 2).

Table 2. Composition of semi synthetic diet of *Helicoverpa armigera*

Sl. No	Component	Quantity
1	Chickpea seeds	100 g
2	Agar- agar (Loba Chemie)	12.8 g
3	Yeast (Loba Chemie)	30 g
4	Methyl parabenzoate (Himedia)	2 g
5	Sorbic acid (Himedia)	1 g
6	Ascorbic acid (Himedia)	3.2 g
7	Streptomycin sulphate (Himedia)	40 mg
8	Vitamins(Multivitamin Tablet)	2 ml
9	Formaldehyde (40%)(Loba Chemie)	1 ml
10	Carbendazim (Bavistin®)	500 mg
11	Water (Distilled)	750 ml

Chickpea seeds (100 g) soaked overnight in 375 ml hot water were blended with 30 g of yeast. Agar, 12.8 g was melted simultaneously in 375 ml of water. After fine grinding of chickpea seeds, molten agar and other ingredients were added into the blender and mixed thoroughly. The prepared diet was poured into sterilized Petri plates and allowed to solidify. The Petri plates containing semi synthetic diet were stored in refrigerator (Kelvinator™) at 4^0C.

3.1.2.2. Rearing of *Helicoverpa armigera*

The semi synthetic diet prepared was not immediately fed to *H. armigera* larvae. The ripened diet after two days of preparation was fed to larvae. Rectangular block of semi synthetic diet weighing 2.5 g was cut with a sterilized knife and transferred into clean and sterilized mutlicavity tray. The *H. armigera* larvae collected from different crops were transferred into trays with the help of camel hair brush (Camlin), a piece of polythene wrapper was kept above and tightened with cover of the tray. Each cavity containing one larva each was assigned with number. The multicavity trays containing larvae were kept at room temperature inside the rearing chamber made up of aluminum mesh to avoid the incidence of parasitic flies belonged order Diptera. The larvae were transferred into a fresh multicavtiy tray with block of semisynthetic diet (2.5 g) once in two days till the pupation. The one day old *H. armigera* pupa was transferred into sterilized multicavity trays, the assigned number for larva and the date of pupation was noted on the cover of the tray. The pupae were taken for sex determination and morphological characterization. The sex of the pupa was noted over the cover of tray. The pupae were allowed for adult emergence and these were used for morphological characterization.

A pair of male and female pupae were transferred into a sterilized Petri plate and placed inside the insect rearing chamber for adult emergence. A black muslin cloth was cut according to dimension and placed inside the rearing chamber as a substrate for oviposition. A cotton swab soaked in 10 per cent honey was kept inside the chamber as feed for the adult moths. The mated female moth laid eggs on the muslin cloth and the neonates emerged after 3-5 days of oviposition were transferred into a Petri plate containing semisynthetic diet till it became second instars. The second instars were then transferred into multivcavity trays to avoid cannibalism and the process of rearing was continued as above.

3.1.3. Morphological characterization

3.1.3.1. Confirming the of identity of species

Morphological characters *viz.*, setal arrangement on prothoracic segment of larva and genitalia structure of both male and female adult moths were studied to confirm the identity of species**.**

3.1.3.1.1. Setal arrangement on prothoracic segment of larva

Ten number of fifth instar larvae from above culture were selected and immobilized them by exposing to ethyl acetate (100%). The killed larvae were then transferred into glass vials containing ethyl alcohol (95%) to preserve the same for long period. The preserved specimen was transferred onto a glass slide and the position of setae on prothoracic segment was observed through stereo binocular microscope (Labomed®). The image of setae on prothoracic segment was captured using microscope with image analyser software (Leica®) and compared with setal map and diagnostic key of *Helicoverpa armigera* developed by Amate *et al*. (1998).

3.1.3.1.2. Adult male and female genitalia structures

Both the male and female adult moths were killed by exposing to ethyl acetate (100%). The abdomen of adult male moth of *Helicoverpa* was detached with blunt forceps and dropped in ethyl alcohol (95%) for one minute and transferred into a labelled test tube containing 10 per cent potassium hydroxide (KOH). It was boiled for 10-20 minutes using spirit lamp and specimen was allowed to cool for 10-20 minutes. The softened abdomen was placed in a Petri dish containing distilled water to remove KOH.

The soft abdominal skin was cleared out with stainless steel needles. Base of the abdomen was held with straight forceps and pressed gently with round end of curve tipped forceps from base to apex and extruded the entire genitalia. This

process was carried out carefully without damaging the aedeagus. The genitalia was placed on a glass slide with drops of water and the genital valves were stretched out with the help of needles, the *harpes* were opened using micro pins to obtain a full face view of inner structures. To examine the aedeagus, the valves were removed first by holding the aedeagus at the base with one set of forceps and pulled both the valves together with the other forceps, thus the valves from the aedeagus were detached.

The genitalia were stained with acid fuschin for two minutes and the excess stain was removed. Drops of Canada balsm were added above the genitalia and xyline was used to remove the air bubble trapped inside the mountant. Cover slip was placed on the slide gently and the glass slide was allowed to dry for one to two days at room temperature, sealed the sides of cover slip with nail polish. The prepared glass slide of genitalia was used for microscopic study.

Compared to the dissection of male genitalia, female genitalia required somewhat different treatment in order to have the internal parts get exposed. When the female abdomen was in Petri dish containing water, the softened abdominal skin between 7th and 8th segment was removed with needles and utmost care was taken no to tear the bursae. The extruded female genitalia was placed on a glass slide with drops of water, subsequently the staining and slide preparation steps were followed similar to that of male genitalia.

3.1.3.2. Morphometry of larval morphotypes of *Helicoverpa armigera* on different crops

The larvae collected from different crops were grouped into larval morphotypes based on shades of colour, markings and longitudinal stripes on larval body and fifth instar larvae from each group were observed for morphometric characters.

3.1.3.2.1. Larval traits

a. Larval colour

Fifty fifth instar larvae from each cluster were observed under natural light and recorded the body colour. Number of larvae belonging to each cluster was recorded and frequency (%) of larval colour morphs occurring in each crop was worked out.

b. Lateral banding pattern

Relative frequency (%) of larvae with pigmentation patterns on lateral bands in population of *H. armigera* occurring in various crop ecosystems were worked out. It was grouped into continuous (%), discontinuous (%) and nil (%) based on observation under microscope with image analyser (Leica^{TM}).

c. Intensity of black pigmentation on thorax and abdomen

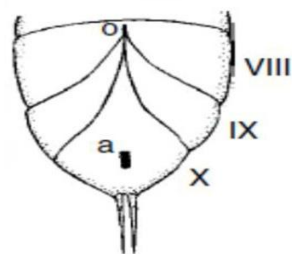
Visual observation on black pigmentation on thoracic and last abdominal segment was recorded and the extent of pigmentation was computed by scoring on 0-4 scale, where '0' = no pigmentation, '1'= lightly pigmented, '2'= moderately pigmented, '3'= slightly deep pigmented and '4'= the deepest pigmentation based on observation under microscope with image analyser (Leica^{TM}).

d. Length, width, weight of larva and width of head capsule

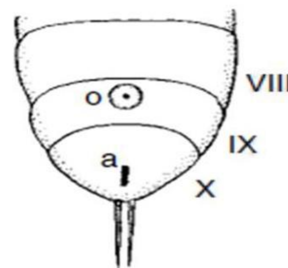
Fifty fifth instar larvae from each cluster were observed under microscope with image analyser (Leica^{TM}) and recorded length, width of larvae and width of head capsule and expressed in millimetre (mm).Weight of larvae from each cluster was taken using weighing balance (Shimadzu^{TM}) and expressed in milligram (mg).

3.1.3.2.2. Pupal traits

Sexing of pupae from each cluster was done by observing the characters of last three abdominal segments. In male pupa, two openings were observed, 1st on 8th and 2nd on 10th abdominal segments whereas in female pupa 1st opening on 9th segment was surrounded by pad like structure and 2nd on 10th abdominal segment.



Male pupa



Female pupa

a. Length, Width and Weight of pupa (mg)

Length and width of pupae were measured using microscope with image analyser (Leica™) and expressed in millimetre (mm) and pupal weight was recorded with the help of micro balance (Shimadzu™) and expressed in milligram (mg).

3.1.3.2.3. Adult traits

Pupae from each cluster were allowed to emerge in a plastic box with cavities and measurements on adult traits were recorded using microscope with image analyser (Leica™).

a. Length and Width of the fore wing

Twenty five moths were observed for recording the length of forewing and width of forewing (perpendicular of costa to anal margin) using the microscope with image analyser (Leica™) and expressed in millimetre (mm).

b. Length of fore, mid and hind femur

The fore, mid and hind legs of adult moths were separated out from the body with forceps and the length of fore, mid and hind femur was recorded using the microscope with image analyser (Leica^{TM}) and expressed in millimetre (mm)

3.1.4. Statistical analysis

The data on morphometric parameters of larvae, pupae and adults of *H. armigera* was analysed by ANOVA single factor with replications method using statistical package (SPSS) Version 16.

3.2. Molecular characterization of *Helicoverpa armigera* larval morphotypes

3.2.1. Isolation of larval genomic DNA

Protocol for genomic DNA isolation from *H. armigera* larva was standardized in the Centre for Plant Biotechnology and Molecular Biology (CPBMB) and All India Network Project on Agricultural Ornithology (AINPAO) laboratory, College of Horticulture, Vellanikkara.

The genomic DNA of the larva was isolated using modified cetyl trimethyl ammonium borate (CTAB) method (Milligan, 1998). For the isolation of genomic DNA, the fifth instar *H. armigera* larvae reared in the multi cavity trays with plant parts were used. From each larval morphotypes, a single larva was transferred in to a Petridish and kept in deep freezer (-20^{0}C) for 30 minutes to kill them. The killed larva was washed in double distilled water and the weight of individual larva was measured using weighing balance (Shimadzu^{TM}). The genomic DNA of the larva was isolated using modified cetyl trimethyl ammonium borate (CTAB) method (Milligan, 1998). Reagents are given in Appendix II.

3.2.1.1. Protocol

The killed larva was ground in a pre chilled mortar and pestle in presence of 500 µl pre-warmed modified CTAB extraction buffer (2X). The homogenised sample was transferred into an autoclaved 1.5 ml Eppendorf tube. The contents were mixed well and incubated at 65^0C for 1 h with occasional mixing by gentle inversion. An equal volume of chloroform: isoamyl alcohol (24:1) was added and centrifuged at 6000 rpm for 15 min. at 4^0C. The supernatant was collected in a fresh 1.5 ml Eppendorf tube, added 40 µl of sodium acetate (3M) and 600 µl of ethanol (95%), incubated in a deep freezer at -20^0C for 20 min and centrifuged at 8000 rpm for 10 min. at 4^0C and the genomic DNA pellet was precipitated out. The DNA pellet was washed with ethanol (70%) by centrifugation at 8000rpm for 10 min. The DNA pellet was air dried for 15 min, dissolved in 25 µl of autoclaved distilled water and stored in deep freezer (-80^0C) for future use.

3.2.1.2. Agarose gel electrophoresis

Agarose gel electrophoresis was performed based on the method described by Sambrook *et al.* (1989) to check the quality of the DNA. Reagents are given in Appendix III.

Procedure

Agarose gel electrophoresis was carried out in electrophoresis unit (BioRad®) and the steps followed are given below.

Agarose (0.8% - 0.8 g in 100ml) solution was prepared in a conical flask with the addition of 1.2 ml 1X TAE buffer. It was kept in LG make micro wave oven for 45 to 60 sec. until agarose was completely dissolved and the solution was clear. The solution was allowed to cool to about 42 to 45^0 C and ethidium bromide (2µl) was added and mixed well. The solution was poured into the gel casting tray (which was placed on a horizontal surface and comb was placed properly in gel caster) and

allowed to solidify for about 30 to 45 min. at room temperature. The comb was removed gently and placed the gel casting tray in the buffer tank, and submerged (just until wells were submerged) with electrophoresis buffer (1X TAE).

DNA Sample was prepared by mixing 1 µl of tracking dye with 5µl of DNA solution. The samples were mixed well and loaded @ 6µl per well and the DNA ladder (100 bp) was loaded in the first lane as a molecular weight marker. The cathode and the anode of the electrophoresis unit were connected to the power pack and the gel was run at constant voltage of 70 volts until the dye migrated to two third length of the gel.

3.2.1.3. Gel documentation

The gel containing electrophoresed DNA was viewed under UV transilluminator for presence of DNA. The DNA fluorescence was observed under UV light due to ethidium bromide dye. The image was documented on gel documentation system (BioRad Gel DOC-It^{TM} imaging system). The gel profile was examined for intactness, clarity of DNA and contamination with RNA and protein.

3.2.1.4. Purity of DNA

The purity of DNA was checked using Nano Drop spectrophotometer model NanoDrop-1000 (Thermo Scientific^{TM}). Nucleic acid shows absorption maxima at 260 nm whereas proteins show peak absorbance at 280 nm. Absorbance has been recorded at both wavelengths and the purity was indicated by the ratio OD_{260}/OD_{280}. A value between 1.8 and 2.0 indicated that the DNA was pure and free from proteins and RNA. When the ratio is <1.8, the sample is contaminated with RNA and the ratio is >2.0 the sample is protein contaminated.

3.2.2. DNA barcoding of tomato fruit borer

To confirm the identity of species of *Helicoverpa* occurring on tomato, DNA barcoding was carried out with following steps.

3.2.2.1. Polymerase Chain Reaction (PCR) with DNA barcode primer and sequencing

Good quality genomic DNA (50 ng/µl) isolated from tomato fruit borer larva was used for DNA barcoding. The universal barcode primer [Hebert *et al*. (2003)] specific to mitochondrial cytochrome oxidase I (mtCO1) was used for PCR amplification. The mtCO1 region was amplified by polymerase chain reaction from genomic DNA using the universal barcode primers (**F**: HCO - 5TAAACTTCAGGGTGACCAAAAAATCA -3', **R:** LCO - 5' - GGTCAACAAATCATAAAGATATTGG -3) in Veriti Thermal Cycler (Applied Biosystems®). The PCR reaction was performed using 5 µl template DNA (50 ng), 0.5 µl of the forward and reverse primers, 0.5 µl of 10 mM dNTP(Genei®), 0.2 µl of Taq DNA polymerase (Genei®), 2.5 µl of Taq DNA buffer B(Genei®), 0.7 µl of $MgCl_2$ and 14.5 µl of Millipore® water. The PCR conditions were programmed as, Lid temperature 98^0C, initial denaturation 94^0C for 5 min, 40 cycles each of denaturation 94^0C for 45 seconds, primer annealing 55^0C for 45 sec and primer extension 72^0C for 45 sec, followed by 10 min extension at 72^0C and storage at 4^0C. The amplified PCR product was run on agarose (1%) gel electrophoresis and the product was sent for sequencing at SciGenom labs, Cochin.

3.2.2.2. Sequence analysis and submission to GenBank, NCBI and Barcode of Life Database (BOLD)

The sequence generated from this study was analyzed for sequence homology using the nucleotide BLAST at NCBI, submitted to BankIt, GenBank and the accession numbers were generated. Further the specimen details and sequences were submitted to BOLD database and barcode for *H. armigera* was generated.

3.2.3. Molecular markers used for the study

Simple Sequence Repeats (SSR) markers were used in the present study. The genomic DNA isolated from twenty two larval morphotypes of *H. armigera* was amplified with the selected eight SSR primers. The amplification pattern for all the larval colour morphs with a specific primer was used to assess the genetic variability existing among them.

3.2.3.1. Standardization of polymerase chain reaction (PCR) conditions

The PCR conditions required for effective amplification of SSR markers included appropriate proportions of the components of the reaction mixture. The reaction mixture included template DNA, assay buffer A with $MgCl_2$, Taq DNA polymerase, dNTPs and forward and reverse primers. The aliquot of this master mix were dispensed into 0.2 ml PCR tubes. The PCR was carried out in Veriti Thermal Cycler (Applied Biosystems^{TM}).

The temperature gradients were set to find out the optimum annealing temperature for the PCR. Annealing temperatures in the range of 52.8 to 57 ^{0}C were tested for 13 SSR primers. Different template concentrations of 20 ng/µl, 25 ng/µl, and 50 ng/µl were used for the standardization of PCR. The thermal cycler was programmed for desired number of cycles and temperatures for denaturation, annealing and polymerization. The amplicons were electrophoresed in two per cent agarose gel, documented and compared with 100 bp DNA ladder (Genei^{TM}, Bangalore).

3.2.3.2 Screening of SSR Primers and analysis

SSR primers supplied by Sigma-Aldrisch, USA were used for amplification of DNA and their sequences were listed in Table 3. These SSR primers were selected as per the high PIC values reported in the previous studies (Subramanian and

Mohankumar, 2006). Thirteen SSR primer combinations were screened by PCR. The amplified products were run along with marker (100bp ladder) on two per cent agarose gel using 1X TAE buffer and stained with ethidium bromide. The image was documented on gel documentation system (BioRad Gel DOC-It™ imaging system). The documented SSR profiles were carefully examined for the polymorphism in banding pattern among larval morphotypes.

3.2.3.3. SSR (Simple Sequence Repeat) analysis

Good quality genomic DNA (25 to 50 ng/µl) isolated from *H. armigera* larval morphotypes was used in the SSR analysis. The amplification was carried out in Veriti Thermal Cycler (Applied Biosystems®). PCR amplification was performed in a 20 µl reaction mixture which consisted of,

a) Genomic DNA (25 ng)	-	2.0 µl
b) 10X Taq assay buffer A	-	1.5 µl
c) dNTPs mix (10mm each)	-	1.5 µl
d) Taq DNA Polymerase (1U)	-	0.3 µl
e) Forward Primer (10pM)	-	2.0 µl
f) Reverse Primer (10pM)	-	2.0 µl
g) Autoclaved Distilled Water	-	10.7 µl
Total volume	-	20.0µl

The thermal cycling was carried out with the following programme

Table 3. Details of Simple Sequence Repeat (SSR) primers used in the study

Sl. No.	Primer	Nucleotide Sequence	Ta (^{0}C)
1	HaSSR1	**F** 5'- TAGGTGATTGTGGCTCAGTTTT-3' **R** 5'- CAAACCCATCAGCAAATGCAAC-3'	57
2	HaSSR2	**F** 5'- AACACCCATTGAAGTCCCATGAA-3' **R** 5'- TTCCTATGTTCACTGCTAGTT-3'	53
3	HaSSR3	**F** 5'- ATCCTTATGCTTTTAGCCGTTTA-3' **R** 5'- CAGTGGACTGCTATAGGCTGA-3'	57
4	HaSSR4	**F** 5'- TGTTACTTGGGTTTCCTGAATA-3' **R** 5'- ACCACCGACACGTGCCGACTTC-3'	55
5	HaSSR5	**F** 5'- GATAAGTTATTTCGGTTTAGTATT-3' **R** 5'- AAGTACCTAATCCGTTTTTATTC-3'	53
6	HaSSR6	**F** 5'- CATAGGAAGTGGTGAAGGGT-3' **R** 5'- CACATTCGTCTTTCATCGAC-3'	53
7	HaSSR7	**F** 5'- ACGTCGATGAAAGACGAATGTGA-3' **R** 5'- AAGCTGGTCTGTGCTGCCAT-3'	57
8	HaSSR8	**F** 5'- GCCGTAATGCCCTCAATTCTT-3' **R** 5'- TTCCCTCGGAGAGCCGT-3'	51
9	HaSSR9	**F**5'- TAGTCTGGGAATTTTGTCTGGTGT-3' **R**5'- CGTGCCATTGAAATAGTAAGCCAT-3'	53
10	HaSSR10	**F**5'- TAAGTATGCCCTCGACTGTCGT-3' **R**5'- CACTTTCCAATTAGCCTCGATGCT-3'	61
11	HaD47	**F**5'- TCAAACACACATACTTGACTA-3' **R**5'- TCCAGCAGTGGAATGCGA-3'	51
12	Hac14	**F** 5'- TCCACACAGTTTGCATTATGA-3' **R** 5'- CGCCATAATCCTATTGATTC-3'	53
13	HaC87	**F** 5'- ACGCGAGCACCAACTGTAA-3' **R** 5'- GAGACCAATAGCAGTAGTTC-3'	45

(F- Forward primer, R- Reverse primer, Ta – Annealing temperature)

Initial denaturation	-	94^0C for 4 minute	
Denaturation	-	94^0C for 1 minute	35 cycles
Primer annealing	-	52.8 ^0C to 57^0C for 1 minute	
Primer extension	-	72^0C for 1 minute	
Final extension	-	72^0C for 5 minutes	
Incubation	-	4^0C for infinity to hold the sample	

3.2.3.4. Native polyacrylamide gel electrophoresis (PAGE)

Reagents

1. **30% Acrylamide** (100ml)
   a) Acrylamide - 29g
   b) Bis-acrylamide - 1g
   c) Distilled water - 100ml

Filtered through 0.45µm filter and stored in brown bottle at 4 ^{o}C.

2. **10% Ammonium per sulphate (APS)**
   a) APS - 100mg
   b) Double distilled water- 1 ml

It should be prepared freshly

3. **10X TBE**
   a) Tris Base - 108g
   b) Boric Acid - 55g
   c) EDTA - 7.45g

Made up the volume to 1 liter with double distilled water

Filtered through 0.45 µm and stored at room temperature

4. **Gel loading dye**

Procedure

- Glass plates and spacers were cleaned thoroughly with distilled water a. The glass plates were then rinsed with sterile distilled water and ethanol (90%) and kept them aside to dry.
- The glass plates and spacer were assembled in the gel caster.
- Poly acrylamide gel solution (8%) was prepared the according to the quantity given below.

Reagents

30% Acrylamide: Bis (29:1)	6.4 ml
5 X TBE	4.8 ml
Distilled H_2O	12.8 ml
Ammonium per sulphate	400 µl
TEMED	20 µl

- Immediately after the preparation, the gel mixture was poured slowly into the previously set glass plate spacer assembly, until the liquid level reaches the top of the upper glass plate.
- Then the comb was inserted between the two glass plates and clamped in place with a small spring clip.
- The remaining acrylamide solution was used to fill the gel mold completely and made sure that no acrylamide solution was leaking from the gel mold. The acrylamide solution was allowed to polymerize for 30 - 40 min.
- Up on the completion of polymerization, the comb was removed by gently wriggling it and lifting out.
- The unpolymerized acrylamide out of the wells was washed by squirting with distilled water.
- The casted gels were transferred into electrophoretic tank and its lower and upper chamber was filled with 1 X TBE.

- The PCR amplified samples were mixed with gel loading dye and loaded in the wells and connected the electrodes to power pack to start the electrophoresis run.
- When the marker dye migrated to desired distance of the gel (~2 h), turned off the electric power and took out gel caster with glass plate- space assembly.
- Detached the upper glass plate smoothly from spacer plate and the gel was stained with 0.2 per cent silver nitrate solution**.**

3.2.3.5. Silver staining protocol

Reagents

Fixer solution

a) Acetic acid - 0.5 ml
b) Ethanol - 10 ml
c) Distilled water - 100 ml

The above solution was mixed well and stored at room temperature

Staining solution (0.2 per cent Silver nitrate)

Silver nitrate (0.2 g) was dissolved in 100 ml of distilled water and stored in brown colored bottle at room temperature.

Developer Solution

a) Sodium hydroxide - 1.5 gm
b) Formaldehyde - 0.5 ml
c) Distilled water - 100 ml

Procedure

- Acrylamide gel was removed from glass plate after the electrophoresis run and transferred in fixer solution for 5 minutes.
- The gel was washed for three times with sterile distilled water.

- Immediately transferred the gel in 0.2 per cent silver nitrate solution and kept for 5 minutes.
- After the 5 minutes, the gel was quickly washed with distilled water for three times.
- Then the gel was transferred in developer solution till the clear bands appear.
- After the development of clear bands, staining procedure was stopped by transferring the gel in 10 per cent acetic acid solution for 10 minutes.
- Then gel was transferred in distilled water and kept for 5 min. The image of gel was captured using gel documentation system (BioRad Gel DOC^{TM}-EZ imaging system).

3.2.3.5. Generation of DNA fingerprints

DNA fingerprint of each larval morphotypes of *H. armigera* occurring on tomato, okra, chickpea and amaranthus was generated based on the presence of clear and distinct bands and size of the bands. Separate colour codes were given to highlight the presence of unique bands, bands shared with two colour morphs, three colour morphs etc. In fingerprints generated for *H. armigera* larval morphotypes occurring on tomato, the presence of unique band was represented in red colour. Violet was used to highlight the bands shared with two larval morphotypes, grey for bands shared with three larval morphotypes, orange for bands shared with four larval morphotypes, yellow for bands shared with five larval morphotypes and green for bands present in all the seven larval morphotypes. Similarly, the bands shared among all larval morphotypes occurring on okra, amaranthus were indicated in green colour, whereas the bands shared among colour morphs were indicated with same colour code used for larval colour morphs occurring on tomato.

3.2.3.6. Data Analysis

Scoring of bands on PAGE gel was done with the ‘Quantity One’ software. λDNA marker (*Eco*RI+*Hind* III double digest) 50bp and 3 kb ladders were used as

molecular weight size marker for each gel along with DNA samples. The bands were scored as one and zero for the presence and absence respectively and their size recorded in relation to the molecular weight markers used and with the software Quantity One. The scored marker data matrix was analyzed using the standard procedure in NTsys Pc 2.0 package (Rohlf, 1998) and the genetic distance or similarity was determined using the Dice coefficient (Dice, 1945). A dendrogram was constructed after cluster analysis of the similarity coefficients by the un-weighted pair-group method analysis, UPGMA (Sneath and Sokal, 1973) using NTsys Pc 2.0 package.

3.2.4. Gut metagenomics of larval *Helicoverpa armigera*

3.2.4.1. Isolation of metagenomic DNA from *Helicoverpa armigera* larval gut

Direct method of isolation of metagenomics DNA described by Zhou *et al.* (1996) was modified and adopted to isolate gut metagenomic DNA from the individual larva. The entire gut was homogenized in 400 µl of extraction buffer [200 mM Tris-HCl (pH-8.0), 25 mM EDTA (pH-8.0), 250 mM NaCl, SDS (0.5%)] in a 1.5 ml Eppendorf tube and spun at 6000 rpm for 10-15 min. The homogenized sample was incubated at room temperature for 1 h. The sample was centrifuged at 12,000 rpm for 5 min. and the supernatant was collected in a fresh Eppendorf tube. An equal volume of phenol: chloroform: isoamyl alcohol (24:25:1) was added to the supernatant and centrifuged at 10000 rpm for 20 min at 4^0C. The aqueous phase was pipetted out into a fresh Eppendorf tube; an equal volume of iso propanol was added, the mixture was incubated at room temperature for 15 min, centrifuged at 13000 rpm for 5 min at room temperature and the metagenomic DNA pellet was precipitated out. The DNA pellet was washed with ethanol (95 %) by centrifugation at 10000 rpm for 10 min. The DNA pellet was air dried, dissolved in 25 µl of autoclaved distilled water and stored in deep freezer (-80^0C) for future use.

3.2.4.2. Quality checking of metagenomic DNA

The 16S rDNA fragment was amplified by polymerase chain reaction from the gut genomic DNA using the universal 16S rDNA primers (**F**- 5' GAGTTTGATCCTGGCTCAG-3', **R**-5'-ACGGCTACCTTGTTACGACTT-3) in Veriti Thermal Cycler (Applied Biosystems®). The PCR reaction was performed using 0.2 µl template DNA (1:50 diluted sample), 0.1 µl of the forward and reverse primers, 1 µl of 10 mM dNTP(Genei®), 0.2 µl of Taq DNA polymerase (Genei®), 2.5 µl of Taq DNA buffer (Genei®) and 15.9 µl of Millipore® water. The PCR conditions were, Lid temperature 98^0C, initial denaturation 94^0C for 2 min, 29 cycles each of denaturation 94^0C for 45 seconds, primer annealing 55^0C for 1 min and primer extension 72^0C for 2 min, followed by 10 min extension at 72^0C and storage at 4^0C. The reaction product was separated on agarose (0.8%) gel to check the quality of bands.

3.2.4.3. 16S ribosomal RNA Amplicon Sequencing using Next Generation Illumina MiSeq™

The metagenomic DNA isolated from *H. armigera* larval gut was outsourced for sequencing in Scigenom, Lab Cochin. Amplicon library was prepared with specific primers spanning the hypervariable V3 region of 16S rRNA gene (Fig. 1) and used for sequencing and subsequent classification.

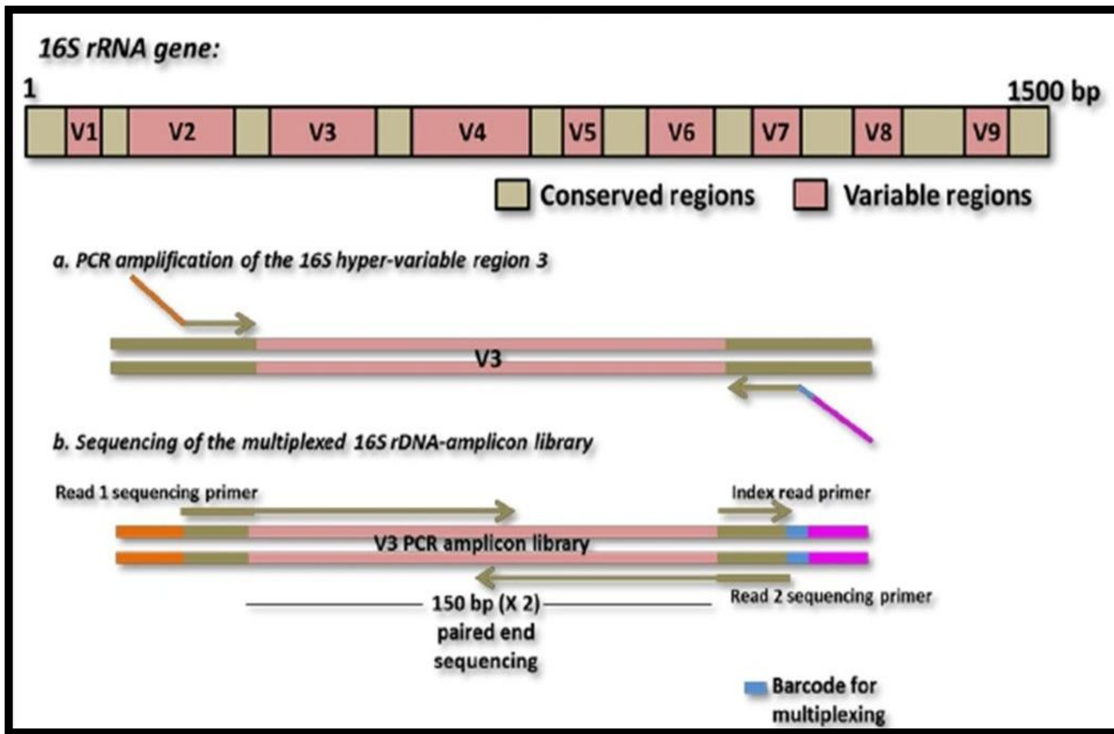


Fig. 1. Multiplexed 16 S rRNA –amplicon sequencing on Illumina MiSeq system

3.2.4.3.1.16S rRNA amplicon library preparation

Amplicon PCR

The extracted gut metagenomic DNA from nine larva was pooled and normalized to 5 ng/µl (purified DNA, 10 mM Tris pH-8.5) and amplicon PCR was carried out using V3 primers (341F 5'CCTACGGGAGGCAGCAG 3',518R 5'ATTACCGCGGCTGCTGG 3'). The PCR master mix consisted of 2 µl each 10 pmol/ul forward and reverse primers, 0.5 µl of 40mM dNTP, 5 µl of 5X Phusion HF reaction buffer, 0.2 µl of 2U/ul F-540 Special Phusion HS DNA polymerase, 5ng input DNA and water to make up the total volume to 25 µL. PCR reaction was programmed, initial denaturation of 98°C for 30 sec, 30 cycles of denaturation 98°C for 10 sec, primer extension of 72°C for 30 sec and final extension at 72°C for 5 min followed by 4°C hold. The PCR product was quantified using the fluorescence quantitative (Qubit 2.0®) fluorometer with the Qubit dsDNA HS assay kit (Invitrogen,USA).

PCR clean-up

PCR clean up was carried out using AMPure XP beads to purify the 16S V3 amplicon away from free primers and primer dimer species. The reagents consisted of 10 mM Tris pH 8.5 (52.5 µl per sample), AMPure XP beads (20 µl per sample), freshly prepared ethanol [EtOH] (80%) (400 µl per sample). Standard protocol was followed and the cleaned up PCR product was stored at -20°C.

Index PCR

Illumina™ Truseq adapters and indices were added (Supplementary table S1) to the cleaned up PCR products. PCR master mix consisted of 2 µl each 10 pmol/ul forward and reverses primers, 1 µl of 40mM dNTP, 10 µl of 5 X Phusion HF reaction buffers, 0.4 µl of 2U/ul F-540 special Phusion HS DNA polymerase, 10 µl (minimum5ng) of PCR1 amplicon and water to make up the total volume to 50 µL.

PCR Reaction was programmed, initial denaturation of 98°C for 30 sec, 15 cycles of denaturation 98°C for 10 sec, primer extension of 72°C for 30 sec and final extension at 72°C for 5 min followed by 4°C hold.

PCR clean-up 2

AMPure XP beads were used to clean up the final library before quantification. The reagents consisted of 10 mM Tris pH 8.5(27.5 µl per sample), AMPure XP beads (56 µl per sample), freshly prepared 80% ethanol (EtOH) (400 µl per sample). Standard protocol was followed and the PCR product was stored at -20°C.

3.2.4.3.2. Library quantification, normalization, and pooling

Libraries were quantified using a fluorometric quantification method and concentrated final library was diluted using distilled water. Diluted DNA (5 µl) from each library was pooled with unique indices.

3.2.4.3.3. Library denaturing and MiSeq sample loading

In preparation for cluster generation and sequencing, pooled libraries were denatured with NaOH, diluted with hybridization buffer, and then heat denatured before MiSeq® sequencing. Each run included a minimum of PhiX (5%) to serve as an internal control for these low diversity libraries. Denatured library was loaded into the reagent cartridge of Illumina MiSeq™ sequencer for sequencing. The output files (fastq) generated from sequencer was used for analysis.

3.2.4.4. Analysis of NGS data

Total raw sequencing reads obtained from sequencer were checked for quality parameters *viz.*, base quality parameters, base composition distribution and GC distribution. After trimming the unwanted sequences from original paired-end data, a consensus V3 region sequence was constructed using Clustal Omega program. Then

we applied multiple filters *viz*., conserved region filter, spacer filter and mismatch filter and the highest quality V3 region sequences were taken for various downstream analyses.

As a part of pre-processing of sequence reads, singletons were removed that were likely due to the sequencing errors and could result in spurious operational taxonomic units (OTUs). This step was achieved by removing the reads that did not cluster with other sequences (abundances <2). Chimeras were also removed using the *de-novo* chimera removal method UCHIME implemented in the tool USEARCH.

Pre-processed reads from all samples were pooled and clustered into OTUs based on their sequence similarity using Uclust program (similarity cutoff = 0.97). QIIME (Caporaso *et al*., 2010) and MG-RAST (Meyer *et al*., 2008) programmes were used for downstream analysis. Representative sequences were identified for each OTU and aligned against Greengenes core set of sequences using PyNAST program (DeSantis *et al*., 2006a; DeSantis *et al*., 2006b). Further this representative sequences were aligned against reference chimeric datasets. Then, taxonomic classification was performed using RDP classifier and Greengenes OTUs database.

The Illumina sequencing data have been submitted to Sequence Read Archive (SRA) of GenBank database as a file under accession number SRR1914365.

Results

4. RESULTS

Results of the investigation on “Identification of larval morphotypes of *Helicoverpa armigera* (Hübner) and their characterization using molecular markers” carried out at Department of Agricultural Entomology, College of Horticulture, Kerala Agricultural University, Vellanikkara are presented hereunder.

4.1. Morphological characterization of *Helicoverpa armigera*

In the present study, *H. armigera* larvae collected from four host plants *viz.,* tomato, okra, chickpea and amaranthus belonged to two major colour phases i.e. green and brown. However, variations observed in the shades of colour, markings and longitudinal stripes on larval body which prompted us to categorize them in to different larval morphotypes. Colour forms and the morphometric parameters of active stages of *H. armigera* were studied in detail to mine out differences existing in the population occurring on different crops. Altogether, twenty two larval morphotypes of *H. armigera* were recorded from four host plants under present investigation.

4.1.1. Confirming the identity of species

Morphological characters *viz.*, setal arrangement on prothoracic segment of larva and genitalia structure of both male and female adult moths of *H. armigera* were studied to confirm the identity of species.

4.1.1.1. Setal arrangement on prothoracic segment of larva

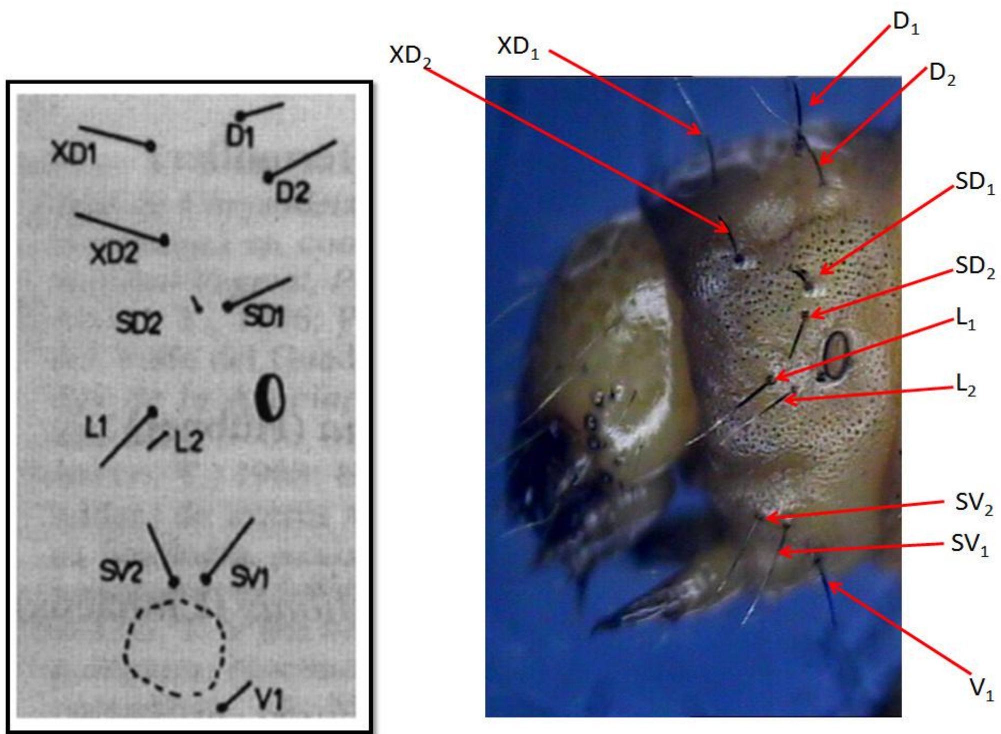
Prothoracic setal arrangement of fifth instar larvae was observed and the image was captured using microscope with image analysing software. A well developed prothoracic shield was present in larva and altogether 11 primary setae were observed on prothorax *viz.*, two dorsal seta (D_1 and D_2), two additional seta (XD_1 and XD_2), two subdorsal seta (SD_1 and SD_2), two lateral seta (L_1 and L_2), two

subventral seta (SV_1 and SV_2) and one ventral seta (V_1). Both the additional setae XD_1 and XD_2 lay near to the anterior margin of prothoracic shield. XD_1 was situated near the mid longitudinal line of the half of shield, while XD_2 near the lateral margin. XD_2 was slightly shorter than XD_1. The dorsal seta D_1 situated posterodorsad to XD_1whereas D_2 posterolaterad to XD_2. The sub dorsal setae SD_1 and SD_2 lied near to lateral margin of prothorasic shield. Two lateral setae L_1 and L_2 lied anterior and horizontally aligned to spiracles, among them L_1 lied laterad to SD_1 and L_2 was identical to SD_2. Subventral setae SV_1 and SV_2 lied above coxa and SV_1 situated anterior to SV_2. The ventral group consisted of a single seta V_1which was situated post coxal and most ventral in position (Plate 1).

4.1.1.2. Adult male and female genitalia structures

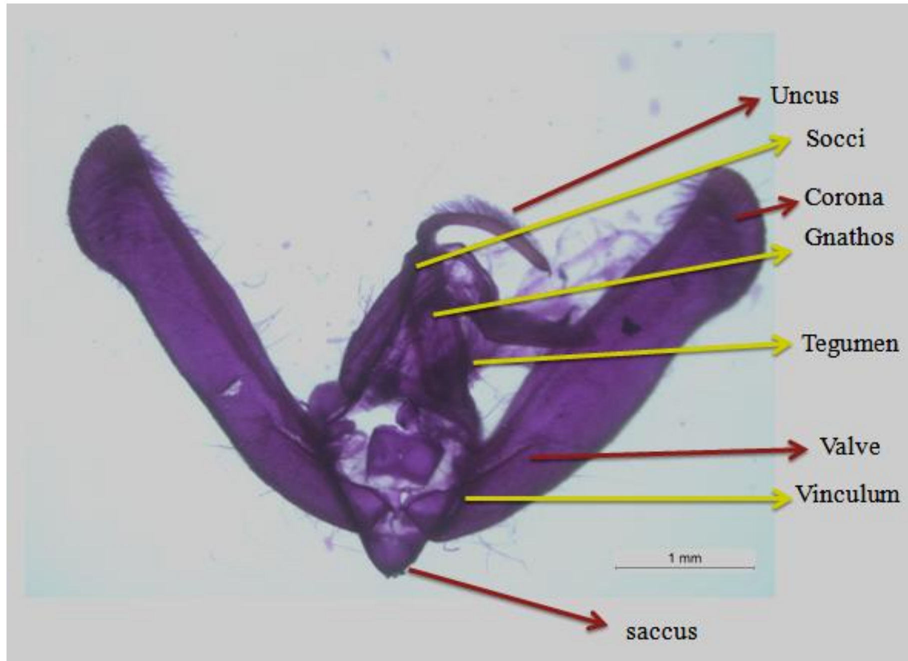
The male genitalia were dissected out from the adult moths and slides were prepared. The parts of male genitalia of adult moth observed were uncus-a hook like structure with hairs arise from caudal end of tegumen, socci- paired organs arising from base of the uncus above gnathos, gnathos-paired organs arise from base of uncus and normally fused at tip into a strong hook, saccus- cephalic portion of vinculum, valves- clasping organs, corona-scleotized spines (Plate 2). In male genitalia, uncus moderately long, well developed, simple, cylindrical, hook like with narrow towards tip; tegumen inverted U shaped; vinculum V shaped, valve long, apically broadened with no projection; corona with numerous closely set seta arranged in several rows; saccus short, stouter with curved apical portion. The aedeagus was elongated, simple, cylindrical, weakly sclerotised structure. The numbers of cornuti (sclerotized spine) inside the aedeagus were 12 (Plate 3a). The long spiral tube occasionally armed with spine called vesica was extended out from the aedeagus (Plate 3b).

The various parts of female genitalia observed were ovipositor, a flattened sclerotized hairy lobe; anterior and posterior apophysis, setae like; ductus bursae



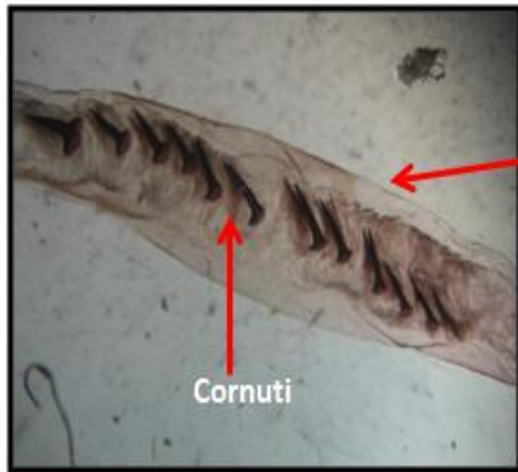
(Setal map, Amate *et al*., 1998) (Magnification 25x)

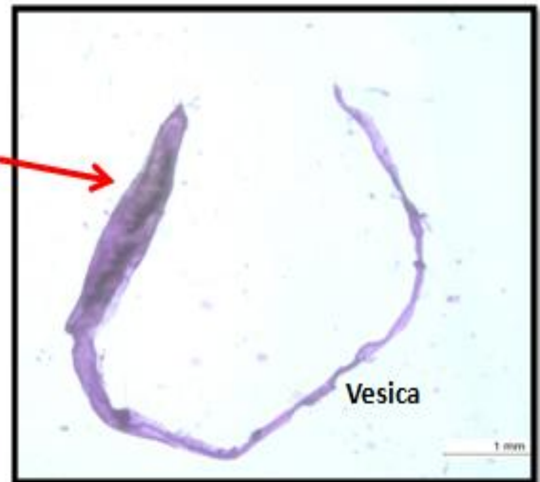
Plate 1. Setal arrangement on prothoracic segment



(Magnification 25x)

Plate 2. Male genitalia of *Helicoverpa armigera*



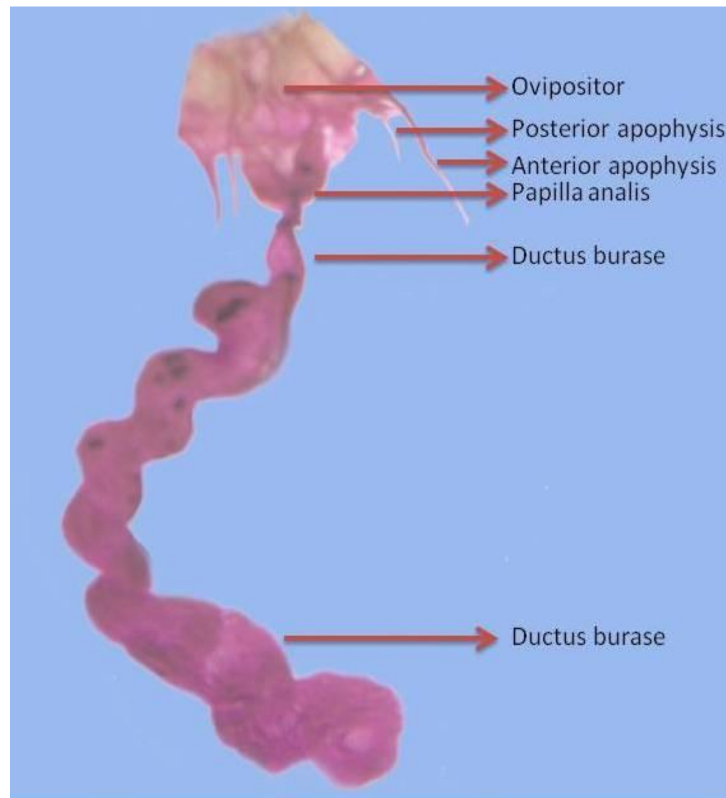


(Magnification 100x)

Plate 3a. Aedeagus and Cornuti

(Magnification 25x)

Plate 3b. Aedeagus and Vesica



(Magnification 25x)

Plate 4. Female genitalia of *Helicoverpa armigera*

duct connecting to bursa copulatix wherein sperms from male deposited during copulation. The ovipositor lobes were well developed, setosed, anterior and posterior apophysis almost of same length, ductus bursae sclerotized towards papilla analis, corpus bursae is oval shaped with 3 signum (Plate 4).

4.1.2. Morphomerty of larval morphotypes of *Helicoverpa armigera* on different crops

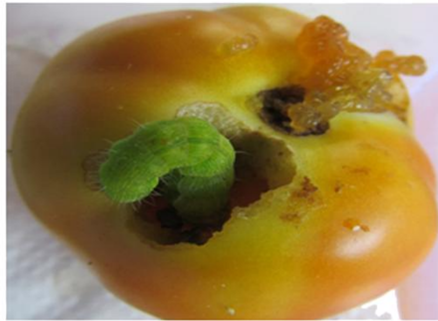
4.1.2.1. Morphometry of larva, pupa and adult of *Helicoverpa armigera* morphotypes on tomato at Palakkad in 2012

Incidence of *H. armigera* was observed in tomato growing area of Palakkad from 33rd to 41st standard week of 2012. Altogether, seven different larval morphotypes *viz*., light green, light green with orange spots, greenish, green with dark green dorsal lines, green with black lines and spot, brown with orange spots and brown with white lateral lines were recorded (Plate 5). The larval parameters *viz*., larval length, width, weight and width of head capsule showed a significant difference among larval morphotypes (Table 4a). The highest larval length recorded in greenish morphotypes (25.97±0.97 mm) followed by light green with orange spots (25.47±1.18 mm). Larvae with light green and brown with white lateral lines had larval length of 25.25±1.02 mm and 25.16±0.70 mm respectively. Green morphotypes with dark green dorsal lines and green with black lines and spots larvae with larval length of 21.99±1.18 mm and 20.50±0.88 mm respectively. However, the lowest larval length was observed in brown with orange spots larva (20.05±0.76 mm).

Greenish morphotypes had the highest larval width (3.70±0.47 mm), followed by light green with orange spots (3.69±0.41 mm), brown with white lateral lines (3.56±0.18 mm), light green (3.55±0.36 mm), green with dark green dorsal lines (3.33±0.32 mm), green with black lines and spots (3.23±0.28 mm). Brown

a. Light green

b. Light green with orange spots



c. Greenish

d. Green with dark green dorsal lines

e. Greenish with black lines and spots

f. Brown with white lateral lines

g. Brown with orange spots

Plate 5. ***Helicoverpa armigera*** **larval morphotypes on tomato**

Table 4a. Morphometry of larva and pupa of *Helicoverpa armigera* morphotypes on tomato (during 33rd to 41st standard week of 2012) at Palakkad

Morphometry of immature stages of *H. armigera*										
Morphotype	Larva				Pupa					
					Male			Female		
	Length (mm)	Width (mm)	Weight (mg)	Width of head capsule (mm)	Length (mm)	Width (mm)	Weight (mg)	Length (mm)	Width (mm)	Weight (mg)
Light green	25.25±1.02	3.55±0.36	272.33±39.12	2.16±0.07	15.27±0.78	3.30±0.24	208.37±39.68	14.64±3.42	3.42±0.27	209.31±43.28
Light green with orange spots	25.47±1.18	3.69±0.41	290.50±57.26	2.13±0.10	15.75±0.60	3.26±0.25	214.29±23.62	15.79±0.59	3.26±0.25	220.75±24.84
Greenish	25.97±0.97	3.70±0.47	303.06±25.70	2.20±0.07	15.67±0.57	3.17±0.24	216.63±21.65	15.86±0.61	3.38±0.31	220.56±21.19
Green with dark green dorsal lines	21.99±1.18	3.33±0.32	231.77±26.28	2.11±0.08	14.28±0.84	3.10±0.20	180.67±34.15	14.57±1.02	3.26±0.25	189.25±26.17
Green with black lines and spots	20.50±0.88	3.23±0.28	227.79±28.36	2.12±0.10	14.55±1.05	3.14±0.24	169.83±25.52	14.76±1.20	3.26±0.28	175.16±24.49
Brown with orange spots	20.05±0.76	3.22±0.25	215.66±16.22	2.11±0.09	14.57±0.51	3.05±0.23	158.15±14.34	14.42±0.63	3.25±0.32	159.94±15.67
Brown with white lateral lines	25.16±0.70	3.56±0.18	298.21±7.66	2.12±0.40	15.66±0.40	3.25±0.27	203.75±22.72	15.75±0.41	3.31±0.37	217.24±21.11
Mean	**23.69±2.52**	**3.50±0.42**	**246.93±48.78**	**2.15±0.10**	**15.15±0.92**	**3.22±0.24**	**197.09±35.69**	**15.17±1.73**	**3.34±0.29**	**201.47±35.75**
CD (p=0.05)	1.09	0.40	38.90	0.09	0.79	0.25	29.84	1.78	NS	29.95

Mean of 50 observations

morphotypes with orange spot had the lowest larval width (3.22±0.25 mm). The mean larval width was 3.50±0.42 mm.

Larval weight was highest in greenish morphotypes (303.06±25.70 mg), followed by brown with white lateral lines (298.21±7.66 mm), light green with orange spots (290.50±57.26 mg), light green (272.33±39.12 mm), green with dark green dorsal lines (231.77±26.28 mg), green with black lines and spots (227.79±28.36 mg). However, the lowest larval weight was observed in brown with orange spots (215.66±16.22 mg).

Width of larval head capsule was the highest in greenish morphotypes (2.20±0.07 mm), followed by light green (2.16±0.07 mm), light green with orange spots (2.13±0.10 mm), brown with white lateral lines (2.12±0.40 mm), green with black lines and spots (2.12±0.10 mm), brown with orange spots (2.11±0.09 mm) and the lowest in green with dark green dorsal lines (2.11±0.08 mm).

Male pupal parameters, pupal length, width and weight showed a significant difference among morphotypes. Highest male pupal length was recorded in larvae with light green with orange spots (15.75±0.60 mm), followed by greenish (15.67±0.57 mm), brown with white lateral lines (15.66±0.40 mm), light green (15.27±0.78 mm), brown with orange spots (14.57±0.51 mm), green with black lines and spots (14.55±1.05 mm), whereas the lowest male pupal length was observed in green with dark green dorsal lines (14.28±0.84 mm).

Width of male pupa was highest in light green (3.30±0.24 mm), followed by light green with orange spots (3.26±0.25 mm), brown with white lateral lines (3.25±0.27 mm), greenish (3.17±0.20 mm), green with black lines and spots (3.14±0.24 mm), green with dark green dorsal lines (3.10±0.20 mm) and brown with orange spots (3.05±0.23 mm).

Male pupa of greenish morphotype had the highest pupal weight (216.63±21.65 mg), which was followed by light green morphotype with orange spots (214.29±23.62 mg), light green (208.37±39.68 mg), brown with white lateral lines (203.75±22.72 mg), green with dark green dorsal lines (180.67±34.15 mg), green with black lines and spots (169.83±25.52 mg), whereas the lowest male pupal weight was observed in brown larvae with orange spots (158.15±14.34 mg).

Female pupal length and weight varied significantly among morphotypes, while a non significant variation was observed in pupal width. Highest pupal length was in greenish larval morphotype (15.86±0.61 mm), followed by light green with orange spots (15.79±0.59 mm), brown with white lateral lines (15.75±0.41 mm), green with black lines and spots (14.76±1.20 mm), green with dark green dorsal lines (14.57±1.02 mm) and the lowest pupal length was recorded in brown with orange spots (14.42±0.63 mm).

Female pupal width was observed highest in light green (3.42±0.27 mm) followed by greenish (3.38±0.31 mm), brown with white lateral lines (3.31±0.37 mm), whereas light green with orange spots, green with dark green dorsal lines and green with black lines and spots recorded mean pupal width of 3.26 mm. However, the lowest female pupal width was observed in brown with orange spots (3.25±0.32 mm).

Light green with orange spots had the highest female pupal weight (220.75±24.84 mg), followed by greenish (220.56±21.19 mg), brown with white lateral lines (217.24±21.11 mg), light green (209.31±43.28 mg), green with dark green dorsal lines (189.25±26.17 mg), green with black lines and spots (175.16±24.49 mg), whereas the lowest pupal weight was observed in brown with orange spots (159.94±15.67 mg).

Adult male moth parameters *viz*., length and width of forewing, length of fore, mid and hind femur varied significantly among morphotypes (Table 4b). Adult moths of greenish morphotype had the highest length of forewing (15.72±0.60 mm), followed by brown with white lateral lines (15.66±0.25 mm), light green with orange spots (15.22±0.88 mm), light green (15.19±0.82 mm), green with dark green dorsal lines (14.88±0.70 mm), green with black line and spots (14.76±0.78 mm), and the lowest length of fore wing was observed in brown with orange spots (14.32±0.43 mm).

Male moths emerged out from green morphotype had the highest width of forewing (6.97±0.45 mm), whereas light green with orange spots recorded the fore wing width of 6.84±0.54 mm, followed by brown with white lateral lines (6.70±0.31 mm), light green (6.66±0.48 mm), green with black lines and spots (6.53±0.48 mm), green with dark green dorsal lines (6.45±0.56 mm), and the lowest width of wing was observed in brown with orange spots (6.45±0.27 mm).

It was observed that both greenish and light green with orange spots had the highest length of fore femur (3.41 mm), followed by light green (3.40±0.05 mm), green with dark green dorsal lines (3.39±0.04 mm), green with black lines and spots and brown with white lateral lines (3.37 mm), whereas the lowest fore femur length of 3.36±0.06 mm was observed in brown with orange spots.

Length of mid femur was the highest in light green and greenish morphotypes (3.81 mm), followed by light green with orange spots (3.79±0.06 mm), green with dark green dorsal lines (3.78±0.05 mm), green with black lines and spots and brown with white lateral lines (3.76 mm)and brown with orange spots (3.71±0.08 mm).

Length of hind femur was observed highest in adults of greenish morphotypes (3.19±0.04 mm), followed by light green (3.18±0.04 mm), light green with orange spots (3.17±0.04 mm) and brown with white lateral lines (3.16±0.04 mm). However,

Table 4b. Morphometry of adult of *Helicoverpa armigera* morphotypes on tomato (during 33rd to 41st standard week of 2012) at Palakkad

Morphotype	Morphometry of *H. armigera* adult moth									
	Male					Female				
	Length of fore wing (mm)	Width of forewing (mm)	Length of fore femur (mm)	Length of mid femur (mm)	Length of hind femur (mm)	Length of fore wing (mm)	Width of fore wing (mm)	Length of fore femur (mm)	Length of mid femur (mm)	Length of hind femur (mm)
Light green	15.19±0.82	6.66±0.48	3.40±0.05	3.81±0.04	3.18±0.04	15.30±0.85	6.73±0.52	3.43±0.05	3.84±0.04	3.20±0.04
Light green with orange spots	15.22±0.88	6.84±0.54	3.41±0.04	3.79±0.06	3.17±0.04	15.40±0.87	6.86±0.52	3.42±0.04	3.80±0.04	3.19±0.03
Greenish	15.72±0.60	6.97±0.45	3.41±0.03	3.81±0.05	3.19±0.04	15.79±0.57	7.05±0.45	3.42±0.03	3.79±0.07	3.19±0.04
Green with dark green dorsal lines	14.88±0.70	6.45±0.56	3.39±0.04	3.78±0.05	3.15±0.04	14.95±0.72	6.50±0.47	3.41±0.02	3.80±0.03	3.17±0.03
Green with black lines and spots	14.76±0.78	6.53±0.48	3.37±0.05	3.76±0.05	3.15±0.04	14.90±0.86	6.58±0.45	3.41±0.33	3.79±0.04	3.18±0.05
Brown with orange spots	14.32±0.43	6.45±0.27	3.36±0.06	3.71±0.08	3.15±0.05	14.47±0.37	6.55±0.27	3.40±0.07	3.74±0.06	3.17±0.05
Brown with white lateral lines	15.66±0.25	6.70±0.31	3.37±0.07	3.76±0.08	3.16±0.04	15.75±0.40	6.83±0.40	3.42±0.04	3.79±0.07	3.18±0.04
Mean	**15.11±0.83**	**6.17±0.04**	**3.39±0.05**	**3.78±0.07**	**3.17±0.05**	**15.24±0.85**	**6.75±0.50**	**3.42±0.04**	**3.79±0.06**	**3.18±0.04**
CD (p=0.05)	0.78	0.51	0.04	0.07	0.04	0.50	0.04	NS	0.06	0.04

Mean of 50 observations

lowest hind femur length of 3.15 mm was recorded in green with dark green dorsal lines, green with black lines and spots and brown with oranges spots.

In female moths, length and width of forewing and length of fore, mid, hind femur varied significantly among morphotypes, however a non significant variation was observed in length of fore femur. Greenish morphotypes had the highest length of forewing (15.79±0.57 mm), followed by brown with white lateral lines (15.75±0.40 mm), light green with orange spots (15.30±0.85 mm), green with dark green dorsal lines (14.95±0.72 mm), green with black lines and spots (14.90±0.86 mm) and the lowest in brown with orange spots (14.47±0.37 mm).

It was observed that the width of forewing was highest in adults of greenish morphotypes (7.05±0.45 mm) followed by light green with orange spots (6.86±0.52 mm), brown with white lateral lines (6.83±0.40 mm), light green (6.73±0.52 mm), green with black lines and spots (6.58±0.45 mm), brown with orange spots (6.55±0.27 mm). However, the green morphotypes with dark green dorsal lines had the lowest forewing width of 6.50±0.47 mm.

Female adult moths of light green morphotype had the highest length of fore, mid and hind femur (3.43±0.05 mm; 3.84±0.04 mm; 3.20±0.04 mm), followed by light green with orange spots (3.42±0.04; 3.80±0.04; 3.19±0.03 mm), whereas the lowest length of fore, mid and hind femur was recorded in adult moths emerged out from brown morphotypes with orange spots (3.40±0.07 mm; 3.74±0.06 mm; 3.17±0.05 mm)

4.1.2.2. Morphometry of larva, pupa and adult of *Helicoverpa armigera* morphotypes on tomato at Thrissur in 2012

In Thrissur, the incidence of *H. armigera* was recorded from 41st standard week and extended up to 44th standard week of 2012. Six different morphotypes *viz*., light green, light green with orange spots, greenish, green with dark green dorsal

lines, green with black lines and spots and brown with orange spots were recorded (Plate 5). Larval parameters *viz*., larval length, weight and width of head capsule showed significant difference among morphotypes whereas, a non significant difference was observed in case of width of larva (Table 5a). Highest larval length was recorded in greenish morphotypes (25.83±0.61 mm), followed by light green with orange spots (25.50±0.70 mm), green with dark green dorsal lines (25.28±0.55 mm), light green (25.06±0.74 mm), green with black lines and spots (21.32±1.11) and lowest larval length was recorded in brown with orange spots (20.75±0.71 mm).

Light green morphotype had the highest larval width (3.45±0.29 mm) succeeded by greenish (3.44±0.39 mm), green with dark green dorsal lines (3.43±0.25 mm), light green with orange spots (3.35±0.05 mm), green with black lines and spots (3.26±0.25 mm) and lowest larval width observed in brown with orange spots (3.25±0.35 mm).

It was observed that the greenish morphotype had the highest larval weight (283.75±41.15 mg), followed by light green (278.09±34.02 mg), light green with orange spots (240.12±27.93 mg), brown with orange spots (231.89±11.65 mg), green with dark green dorsal lines (225.18±19.82 mg) and the lowest larval weight was recorded in green with black lines and spots (218.23±22.57 mg).

Width of larval head capsule was observed the highest in green morphotypes (2.31±0.01 mm), whereas light green with orange spots recorded larval width of 2.24±1.05 mm followed by light green (2.17±0.09 mm), green with dark green dorsal lines and green with black lines and spots (2.16±0.07 mm) and the lowest width of head capsule was recorded in brown morphotypes with orange spots (2.15±0.08 mm).

Male pupal length, width and weight varied significantly among morphotypes. Light green morphotype had the highest pupal length (15.83±0.66 mm), followed by greenish (15.50±0.70 mm), green with dark green dorsal lines (15.38±0.67 mm), light

Table 5a. Morphometry of larva and pupa of *Helicoverpa armigera* morphotypes on tomato (during 41st to 44th standard week of 2012) at Thrissur

Morphometry of immature stages of *H. armigera*										
	Larva				Pupa					
					Male			Female		
Morphotype	**Length (mm)**	**Width (mm)**	**Weight (mg)**	**Width of head capsule (mm)**	**Length (mm)**	**Width (mm)**	**Weight (mg)**	**Length (mm)**	**Width (mm)**	**Weight (mg)**
Light green	25.06±0.74	3.45±0.29	278.09±34.02	2.17±0.09	15.83±0.61	3.14±0.14	213.98±7.18	15.90±0.60	3.70±0.62	202.08±29.54
Light green with orange spots	25.50±0.70	3.25±0.35	240.10±27.93	2.24±1.05	15.33±0.86	3.14±0.25	198.44±29.42	15.83±0.25	3.50±0.52	225.98±1.35
Greenish	25.83±0.61	3.44±0.39	283.75±41.15	2.31±0.01	15.50±0.70	3.13±0.22	224.42±4.20	15.82±0.71	3.61±0.25	226.75±25.21
Green with dark green dorsal lines	25.28±0.55	3.43±0.25	225.18±19.82	2.16±0.07	15.38±0.67	3.11±0.22	196.40±21.12	15.72±0.87	3.33±0.25	198.50±21.12
Green with black lines and spots	21.32±1.11	3.26±0.25	218.23±22.57	2.16±0.07	15.07±0.35	3.10±0.03	196.40±21.21	15.72±0.87	3.17±0.24	198.50±21.12
Brown with orange spots	20.75±0.71	3.35±0.04	231.89±11.65	2.15±0.08	14.88±0.92	3.09±0.05	168.67±21.32	14.97±1.87	3.33±0.25	174.30±21.84
Mean	**24.05±2.06**	**3.40±0.28**	**243.76±40.17**	**2.18±0.08**	**15.57±0.80**	**3.07±0.18**	**200.78±33.21**	**15.66±0.82**	**3.42±1.85**	**203.85±32.09**
CD (p=0.05)	0.93	NS	34.31	0.09	0.90	0.20	31.29	0.96	NS	31.20

Mean of 50 observation

green with orange spots (15.33±0.35 mm), green with black lines and spots (15.07±0.35 mm) and the lowest male pupal length (14.88±0.92 mm) was recorded in brown morphotypes with orange spots.

Highest pupal width was observed in larvae with both light green and light green with orange spots (3.14 mm), followed by greenish (3.13±0.22 mm), green with dark green dorsal lines (3.11±0.22 mm), green with black lines and spots (3.10±0.03 mm) and the lowest male pupal width was seen in brown morphotype with orange spots (3.09±0.05 mm).

Male pupal weight was highest in greenish morphotype (224.42±4.20 mg), subsequently light green (213.98±7.18 mg), light green with orange spots (198.44±29.42 mg), both green with orange spots and green with black lines and spots (196.40±21.12 mg) and brown with orange spots (168.67±21.32 mg) were followed.

Female pupal length was observed highest in light green morphotype (15.90±0.60 mm), followed by light green with orange spots (15.83±0.25 mm), greenish (15.82±0.07 mm). However, both green with dark green dorsal lines and green with black lines and spots had the mean pupal length of 15.72 mm, whereas brown morphotype with orange spots was recorded the lowest female pupal length (14.97±1.87 mm).

It was observed that the light green morphotype had the highest female pupal width (3.70±0.62 mm), followed by greenish (3.61±0.25 mm), light green with orange spots (3.50±0.52 mm), both green with dark green dorsal lines and green with black lines and spots (3.33±0.25 mm), however the lowest pupal width (3.17±0.24 mm) was recorded in brown morphotype with orange spots.

Female pupal weight was observed highest in greenish morphotype (226.73±25.21 mg), succeeded by light green with orange spots (225.98±1.35 mg),

light green (202.08±29.54 mg), green with dark green dorsal lines (198.50±21.12 mg), green with black lines and spots (195.50±21.12 and the lowest pupal weight was recorded in brown morphotype with orange spots (174.30±21.84 mg).

Adult male moth parameters *viz*., length and width of forewing, length of fore, mid and hind femur varied significantly among morphotypes (Table 5b). Length of fore wing was recorded highest in moths of greenish morphotypes (15.72±0.79 mm), followed by light green with orange spots (15.59±0.78 mm), light green (15.41±1.02 mm), green with dark green dorsal lines (15.09±0.07 mm), green with black lines and spots (14.90±0.05 mm) and the lowest length of forewing (14.89±0.67 mm) was observed in brown morphotype with orange spots.

Width of forewing was recorded highest in male moths of greenish morphotype (6.88±0.60 mm), followed by green with dark green dorsal lines (6.75±0.43 mm), light green with orange spots (6.59±0.75 mm), light green (6.56±0.61 mm), green with black lines and spots (6.45±0.49 mm) and brown with orange spots (6.11±0.71 mm).

Length of fore, mid and hind femur was observed highest in greenish morphotype (3.40±0.02 mm; 3.80±0.04 mm; 3.25±0.02 mm), and it was recorded lowest in brown with orange spots (3.39±0.03 mm; 3.79±0.05 mm; 3.20±0.04 mm) with mean length of fore, mid and hind femur of 3.39±0.03, 3.79±0.05 and 3.20±0.04 mm respectively.

Similarly, female moths of greenish morphotype had the highest length (15.79±0.49 mm) and width (7.05±0.58 mm) of forewing, whereas lowest length and width of forewing was observed in brown with orange spots (15.02±0.71 mm and 6.26±0.64 mm).

A non significant variation was recorded in length of fore, mid and hind femur among morphotypes. Highest length of fore, mid and hind femur was observed in

Table 5b.Morphometry of adult of *Helicoverpa armigera* morphotypes on tomato (during 41st to 44th standard week of 2012) at Thrissur

Morphotype	Morphometry of *H. armigera* adult moth									
	Male					Female				
	Length of fore wing (mm)	Width of forewing (mm)	Length of fore femur (mm)	Length of mid femur (mm)	Length of hind femur (mm)	Length of fore wing (mm)	Width of fore wing (mm)	Length of fore femur (mm)	Length of mid femur (mm)	Length of hind femur (mm)
Light green	15.41±1.02	6.56±0.61	3.38±0.02	3.78±0.04	3.21±0.03	15.51±0.96	6.56±0.04	3.41±0.25	3.82±0.04	3.22±0.03
Light green with orange spots	15.59±0.78	6.59±0.75	3.39±0.03	3.78±0.04	3.20±0.04	15.15±0.66	6.55±0.43	3.41±0.02	3.82±0.05	3.24±0.07
Greenish	15.72±0.79	6.88±0.60	3.40±0.02	3.80±0.04	3.25±0.02	15.79±0.49	7.05±0.58	3.41±0.03	3.84±0.09	3.24±0.04
Green with dark green dorsal lines	15.09±0.05	6.75±0.49	3.38±0.03	3.80±0.05	3.20±0.09	15.53±0.62	7.00±0.49	3.40±0.02	3.82±0.04	3.23±0.05
Green with black lines and spots	14.89±0.67	6.45±0.49	3.33±0.03	3.78±0.04	3.19±0.05	15.02±0.71	6.26±0.64	3.40±0.02	3.81±0.04	3.22±0.05
Brown with orange spots	14.90±0.65	6.11±0.71	3.36±0.04	3.79±0.05	3.18±0.05	15.45±1.67	6.79±0.62	3.39±0.03	3.80±0.04	3.20±0.05
Mean	**15.16±0.90**	**6.32±0.64**	**3.37±0.03**	**3.79±0.05**	**3.20±0.04**	**15.45±1.67**	**6.70±0.62**	**3.40±0.02**	**3.81±0.04**	**3.22±0.05**
CD (p=0.05)	0.96	0.71	0.05	0.03	0.05	NS	0.69	NS	NS	NS

Mean of 50 observations

female moths of green morphotype (3.41±0.03 mm; 3.84±0.09 mm; 3.24±0.04 mm), whereas lowest length of fore, mid and hind femur was noted in brown with orange spots (3.39±0.03; 3.80±0.04; 3.20±0.05 mm).

4.1.2.3. Morphometry of larva, pupa and adult of *Helicoverpa armigera* morphotypes on tomato at Palakkad in 2013

During 2013, the infestation of *H. armigera* was noticed on tomato in Palakkad from 35th standard week to 41st standard week. Altogether seven different morphotypes were recorded (Plate 5).

The larval parameters recorded *viz*., larval length width, weight and width of head capsule varied significantly among morphotypes. Green morphotype had the highest larval length (25.85±1.43 mm), followed by light green (25.75±1.09 mm), light green with orange spots (24.95±3.47 mm) brown with white lateral lines (24.66±0.93 mm), green with dark green dorsal lines (21.14±1.06 mm), green with black lines and spots (20.82±0.94 mm), however the lowest larval length was observed in brown morphotype with orange spots (20.56±0.70 mm) (Table 6a).

Green colurmorph had the highest larval width (3.65±0.51 mm), followed by brown with white lateral lines (3.55±0.05 mm), both light green and light green with orange spots (3.47±0.34 mm), green with dark green dorsal lines (3.26±0.25 mm), green with black lines and spots (3.23±0.25 mm) and the lowest larval width was recorded in brown morphotype with orange spots (3.17±0.24 mm).

Larval weight was observed highest in green morphotype (302.03±27.64 mg), followed by brown with white lateral lines (299.04±8.48 mg), light green (296.77±23.40 mg), light green with orange spots (272.73±30.21 mg), green with black lines and spots (225.62±21.61 mg), green with dark green dorsal lines (218.82±20.91 mg) and lowest larval weight recorded in brown morphotype with orange spots (214.34±13.44 mg).

Table 6a.Morphometry of larva and pupa of *Helicoverpa armigera* morphotypes on tomato (during 35th to 41st standard week of 2013) at Palakkad

Morphometry of immature stages of *H. armigera*										
Morphotype	Larva				Pupa					
					Male			Female		
	Length (mm)	Width (mm)	Weight (mg)	Width of head capsule (mm)	Length (mm)	Width (mm)	Weight (mg)	Length (mm)	Width (mm)	Weight (mg)
Light green	25.75±1.09	3.47±0.34	296.77±23.40	2.16±0.09	15.60±0.59	3.17±0.24	213.44±18.78	15.72±0.63	3.25±0.25	217.66±17.22
Light green with orange spots	24.95±3.47	3.47±0.34	272.73±30.21	2.15±0.07	15.70±0.59	3.17±0.24	206.45±33.64	15.75±0.54	3.35±0.28	218.56±24.60
Greenish	25.85±1.43	3.65±0.51	302.03±27.64	2.16±0.09	15.73±0.59	3.21±0.25	227.87±20.26	15.85±0.60	3.35±0.32	220.24±37.35
Green with dark green dorsal lines	21.14±1.06	3.26±0.25	218.82±20.91	2.14±0.08	14.95±0.54	3.18±0.05	176.87±23.74	15.07±0.67	3.33±0.24	200.09±24.97
Green with black lines and spots	20.82±0.94	3.23±0.25	225.62±21.61	2.14±0.12	15.15±6.79	3.12±0.22	161.38±21.37	15.30±0.89	3.25±0.30	196.51±22.49
Brown with orange spots	20.56±0.70	3.17±0.24	214.34±13.44	2.12±0.08	14.70±0.41	3.13±0.17	157.68±11.03	14.96±0.29	3.16±0.25	189.52±11.96
Brown with white lateral lines	24.66±0.93	3.55±0.05	299.04±8.48	2.15±0.03	15.50±0.44	3.07±0.18	209.35±22.13	15.66±0.51	3.26±0.22	215.13±19.51
Mean	**23.27±2.46**	**3.38±0.36**	**259.05±43.81**	**2.15±0.10**	**15.26±0.77**	**3.17±0.24**	**190.46±32.73**	**15.35±1.94**	**3.19±0.28**	**193.54±34.07**
CD (p=0.05)	1.73	0.55	38.23	0.14	0.98	0.36	38.71	NS	NS	40.46

Mean of 50 observations

It was observed that both light green and greenish morphotype had the highest width of head capsule (2.16±0.09 mm), followed by both light green with orange spots and brown with white lateral lines (2.15 mm), both green with dark green dorsal lines and green with black lines and spots (2.14 mm), and lowest width of head capsule was recorded in brown morphotype with orange spots (2.12±0.08 mm).

Male pupal length, width and weight varied significantly among morphotypes. Highest pupal length was recorded in green colurmorph (15.73±0.59 mm), succeeded by light green with orange spots (15.70±0.59 mm), light green (15.60±0.59 mm), brown with white lateral lines (15.50±0.44 mm), green with black lines and spots (15.15±6.79 mm), green with dark green dorsal lines (14.95±0.54 mm) and brown with orange spots (14.70±0.41 mm).

Greenish morphotype had the highest pupal width (3.21±0.25 mm), whereas brown with white lateral lines was recorded pupal width of 3.18±0.05 mm, followed by both light green and light green with orange spots (3.17±0.24 mm), green with dark green dorsal lines (3.13±0.17 mm), green with black lines and spots (3.12±0.22 mm) and lowest pupal width was observed in brown with orange spots (3.07± 0.18 mm).

Highest pupal weight was observed in greenish morphotype (227.87±20.76 mg), followed by light green (213.44±18.78 mg), brown with white lateral lines (209.35±22.13 mg), light green with orange spots (206.45±33.64 mg), green with dark green dorsal lines (176.87±23.74 mg), green with black lines and spots (161.38±21.37 mg) and the lowest male pupal weight was recorded in brown with orange spots (157.68±11.03 mg).

Female pupal length and width showed non significant variation among morphotypes, whereas pupal weight varied significantly. Pupal length, width and

weight was observed highest in greenish morphotypes (15.85±0.80 mm; 3.35±0.32 mm; 230.24±37.35 mg), whereas lowest in brown morphotypes with white lateral lines (14.96±0.29 mm; 3.16±0.25 mm; 189.52±11.96 mg).

Adult male moths of greenish morphotype had the highest length of forewing (15.75±0.67 mm), subsequently brown with white lateral lines (15.66±0.26 mm), green with dark green dorsal lines (15.50±0.72 mm), light green (15.40±0.75 mm), light green with orange spots (14.90±0.98 mm), green with black line and spots (14.72±0.63 mm) and brown with orange spots (14.20±0.23 mm) were followed (table 6b).

Width of forewing was recorded highest in greenish morphotypes (6.87±0.48 mm), and it was observed lowest in green with black lines and spots (6.35±0.59 mm). Length of fore, mid and hind femur was noted highest in greenish colurmorph (3.42 mm; 3.80 mm; 3.19 mm),whereas lowest in brown with white lateral lines (3.38 mm; 3.70 mm; 3.14 mm).

In adult female moths, length and width of forewing was observed highest in greenish morphotype (15.77±0.67 mm and 6.95±0.53 mm), whereas length and width of forewing was recorded lowest in brown with orange spots (14.30±0.45 mm) and green with black lines and spots (6.42±0.59 mm) respectively.

Female adult moths emerged out from greenish morphotype had the highest length of fore, mid and hind femur (3.43±0.03 mm; 3.83±0.04 mm; 3.20±0.04 mm). Whereas, lowest length of fore, mid and hind femur was recorded in brown with orange spots (3.41±0.02 mm; 3.71±0.05 mm; 3.17±0.06 mm).

Table 6b. Morphometry of adult of *Helicoverpa armigera* morphotypes occurring on tomato (during 35th to 41st standard week of 2013) at Palakkad

Morphotype	Morphometry of *H. armigera* adult moth									
	Male					Female				
	Length of fore wing (mm)	Width of forewing (mm)	Length of fore femur (mm)	Length of mid femur (mm)	Length of hind femur (mm)	Length of fore wing (mm)	Width of fore wing (mm)	Length of fore femur (mm)	Length of mid femur (mm)	Length of hind femur (mm)
Light green	15.40±0.75	6.72±0.49	3.40±0.08	3.79±0.05	3.19±0.03	15.60±0.73	6.75±0.55	3.42±0.02	3.79±0.08	3.19±0.03
Light green with orange spots	14.90±0.98	6.57±0.54	3.40±0.04	3.79±0.05	3.17±0.03	15.70±0.59	6.65±0.81	3.42±0.02	3.81±0.04	3.19±0.03
Greenish	15.75±0.67	6.87±0.48	3.42±0.02	3.80±0.03	3.19±0.04	15.77±0.67	6.95±0.53	3.43±0.03	3.83±0.04	3.20±0.04
Green with dark green dorsal lines	15.50±0.72	6.56±0.45	3.39±0.03	3.78±0.04	3.17±0.03	15.19±0.61	6.54±0.40	3.40±0.01	3.80±0.02	3.18±0.04
Green with black lines and spots	14.72±0.63	6.43±0.17	3.39±0.03	3.77±0.05	3.18±0.04	14.90±0.08	6.42±0.59	3.40±0.02	3.79±0.03	3.19±0.04
Brown with orange spots	14.20±0.31	6.35±0.59	3.38±0.05	3.70±0.05	3.14±0.05	14.30±0.45	6.45±0.22	3.41±0.02	3.71±0.05	3.17±0.06
Brown with white lateral lines	15.66±0.26	6.77±0.25	3.41±0.03	3.79±0.05	3.18±0.01	15.85±0.25	6.66±0.25	3.43±0.02	3.71±0.05	3.18±0.04
Mean	**15.06±0.84**	**6.54±0.54**	**3.40±0.04**	**3.78±0.05**	**3.17±0.04**	**15.21±0.81**	**6.61±0.51**	**3.42±0.03**	**3.79±0.08**	**3.19±0.09**
CD (p=0.05)	1.16	0.78	0.07	NS	0.07	1.14	0.79	NS	0.05	0.09

Mean of 50 observations

4.1.2.4. Morphometry of larva, pupa and adult of *Helicoverpa armigera* morphotypes on okra at Thrissur during 2012-13.

Incidence of *H. armigera* was noticed on okra from 41st to 47th standard week of 2012 and 7th to 10th standard week of 2013 at Thrissur. Altogether six different morphotypes *viz.*, light green, light green with orange spots, yellowish green, greenish, brownish, brown with dark brown longitudinal lines were observed (Plate 6). The larval parameters *viz.*, larval length, width, weight and width of head capsule varied significantly among morphotypes (Table 7a). Highest larval length was recorded in yellowish green morphotypes (27.19±0.51 mm), followed by light green (26.25±0.92 mm), light green with orange spots (25.89±0.71 mm), brown with dark brown longitudinal lines (25.62±0.02 mm), greenish (24.45±1.40)and the lowest larval length was noticed in brownish morphotype (24.18±1.08 mm).

Yellowish green colourmoph had the highest larval width (3.93±0.49 mm), followed by light green (3.56±0.38 mm), light green with orange spots (3.42±0.26 mm), brown with dark brown longitudinal lines (3.37±0.25 mm), brownish (3.25±0.26 mm) and Greenish morphotypes (3.22±0.25 mm).

Larval weight was observed highest in yellowish green morphotypes (320.76±28.05 mg), succeeded by light green (288.01±21.15 mg), light green with orange spots (278.64±27.10 mg), brown with dark brown longitudinal lines (274.33±21.44 mg), brownish (252.31±25.79 mg) and greenish morphotypes (249.81±30.20 mg).

Brown with dark brown longitudinal lines had the highest width of larval head capsule (2.23±0.06 mm), followed by yellowish green (2.21±0.03), light green with orange spots (2.19±0.06 mm), and lowest width of head capsule (2.18 mm) recorded in light green, greenish and brownish morphotypes.

a. Light green

b. Light green with orange spots

c. Yellowish green

d. Greenish

d. Brownish

d. Brown with dark brown longitudinal lines

Plate 6. *Helicoverpa armigera* larval morphotypes on okra

Table 7a. Morphometry of larva and pupa of *Helicoverpa armigera* morphotypes on okra (during 41st to 47th standard week of 2012 and 7th to 10th standard week of 2013) at Thrissur

Morphometry of immature stages of *H. armigera*										
	Larva				Pupa					
					Male			Female		
Morphotype	Length (mm)	Width (mm)	Weight (mg)	Width of head capsule (mm)	Length (mm)	Width (mm)	Weight (mg)	Length (mm)	Width (mm)	Weight (mg)
Light green	26.25±0.92	3.56±0.38	288.01±21.15	2.18±0.05	15.83±0.61	3.16±0.35	203.76±22.37	15.91±0.58	3.29±0.32	205.63±23.12
Light green with orange spots	25.89±0.71	3.42±0.26	278.64±27.10	2.19±0.06	15.64±0.49	3.11±0.15	184.05±18.96	15.71±0.42	3.25±0.25	189.29±18.97
Yellowish green	27.91±0.51	3.93±0.49	320.76±28.05	2.21±0.03	16.37±0.55	3.23±0.62	226.04±21.35	16.43±0.57	3.36±0.34	231.68±26.89
Greenish	24.45±1.41	3.22±0.25	249.81±30.20	2.19±0.06	15.25±0.09	3.08±0.71	171.88±36.77	15.37±0.72	3.27±0.29	178.99±36.62
Brownish	24.18±1.08	3.25±0.26	252.31±25.79	2.18±0.08	14.57±0.57	3.09±0.65	170.08±22.17	14.75±0.46	3.25±0.26	175.44±20.33
Brown with dark brown longitudinal lines	25.62±0.02	3.37±0.25	274.33±21.44	2.23±0.06	15.35±0.62	3.11±0.19	175.67±11.09	15.50±0.40	3.12±0.25	177.30±10.99
Mean	**25.95±1.56**	**3.44±o.32**	**266.46±37.04**	**2.20±0.07**	**15.50±0.80**	**3.09±0.25**	**190.11±33.32**	**15.73±0.71**	**3.28±0.30**	**197.91±34.91**
CD(p=0.05)	1.15	0.48	36.14	0.07	0.82	0.32	35.62	0.77	NS	36.72

Mean of 50 observations

Male pupal length was noticed highest in yellowish green colurmorph (16.37±0.55 mm), foloowed by light green (15.38±0.61 mm), light green with orange spots (15.64±0.49 mm), brown with dark brown longitudinal lines (15.35±0.62 mm), greenish (15.25±0.09 mm) and lowest pupal length of 14.57±0.57 mm was recorded in pupa of brownish morphotypes. Highest pupal width recorded in yellwish green (3.23±0.62 mm and lowest in greenish morphotypes (3.08±0.71 mm).

Pupal weight was observed highest in yellowish green morphotypes (226.04±21.35 mg), followed by light green (203.76±22.37 mg) and a lowest pupal weight of 170.08±22.17 mg was recorded in brownish morphotypes.

Yellowish green morphotype had the highest Length of female pupa (16.43±0.57 mm), followed by light green (15.91±0.58 mm), light green with orange spots (15.761±0.42 mm), brown with dark brown longitudinal lines (15.50±0.40 mm), greenish (15.37±0.72 mm) and brownish morphotype (14.75±0.46 mm).

Highest female pupal width was recorded in light green morphotypes and (3.36±0.34) the lowest in brown with dark brown longitudinal lines (3.21±0.25 mm). Yellowish green morphotypes had the highest pupal weight 231.68±26.89 mg), however the lowest pupal weight was noticed in brownish morphotypes (175.44±20.33 mg).

Length and width of forewing and length of fore, mid and hind femur varied significantly among morphotypes. Length of forewing was observed highest in yellowish green morphotype (16.13±0.74 mm), followed by light green (15.87±0.66 mm), light green with orange spots (15.25±0.66 mm), greenish (15.08±0.67 mm), brown with dark brown longitudinal lines (14.85±0.25 mm), whereas lowest length of forewing was recorded in brownish morphotype (14.31±0.59 mm).

Male moths of yellowish green morphotypes had the highest width of forewing (7.07±0.38 mm) and it was recorded lowest in brownish morphotype

Table 7b. Morphometry of adult of *Helicoverpa armigera* morphotypes on okra (during 41st to 47th standard week of 2012 and 7th to 10th standard week of 2013) at Thrissur

Morphotype	Morphometry of *H. armigera* adult moth									
	Male					Female				
	Length of forewing (mm)	Width of Forewing (mm)	Length of fore femur (mm)	Length of mid femur (mm)	Length of hind femur (mm)	Length of forewing (mm)	Width of Forewing (mm)	Length of fore femur (mm)	Length of mid femur (mm)	Length of hind femur (mm)
Light green	15.87±0.66	7.01±0.46	3.38±0.02	3.77±0.04	3.18±0.02	16.04±0.69	7.02±0.45	3.39±0.02	3.79±0.04	3.19±0.01
Light green with orange spots	15.25±0.61	6.78±0.46	3.39±0.02	3.76±0.03	3.18±0.01	15.41±0.61	6.92±0.51	3.39±0.02	3.80±0.04	3.20±0.02
Yellowish green	16.13±0.74	7.07±0.38	3.41±0.03	3.83±0.04	3.23±0.62	16.19±0.68	7.16±0.43	3.42±0.03	3.83±0.03	3.24±0.04
Greenish	15.08±0.67	6.62±0.47	3.38±0.03	3.76±0.04	3.18±0.01	15.77±0.67	6.75±0.48	3.39±0.05	3.80±0.05	3.19±0.02
Brownish	14.31±0.59	6.12±0.23	3.37±0.02	3.79±0.05	3.17±0.02	14.93±0.86	6.77±0.35	3.38±0.02	3.78±0.09	3.17±0.02
Brown with dark brown longitudinal lines	14.85±0.25	6.59±0.47	3.37±0.01	3.79±0.05	3.18±0.06	15.02±0.40	6.77±0.50	3.41±0.03	3.79±0.06	3.19±0.02
Mean	**15.27±0.85**	**6.55±0.52**	**3.39±0.03**	**3.79±0.05**	**3.19±0.03**	**15.46±0.80**	**6.89±0.72**	**3.40±0.03**	**3.80±0.06**	**3.20±0.03**
CD(p=0.05)	0.90	0.59	NS	0.04	0.06	0.92	NS	0.04	0.04	NS

Mean of 50 observations

(6.12±0.23 mm) with a mean width of forewing, 6.55±0.52 mm. Length of fore, mid and hind femur was observed highest in yellowish green morphotype (3.41±0.03 mm; 3.83±0.04 mm; 3.23±0.62 mm) and lowest length of fore, mid and hind femur was noticed in brownish morphotype (3.37±0.02 mm; 3.79±0.05 mm; 3.17±0.02 mm) (Table 7b).

Female moths emerged out of yellowish green morphotype had the highest length of forewing (16.19±0.68 mm), followed by light green (16.04±0.69 mm), greenish (15.77±0.67 mm), light green with orange spots (15.41±0.61 mm), brown with dark brown longitudinal lines (15.02±0.04 mm) and the lowest length of forewing was recorded in brownish morphotype (14.93±0.86 mm).

Width of forewing was observed highest in female adult moths of yellowish green morphotypes (7.16±0.43 mm) and lowest in brownish morphotype (6.77±0.35 mm). Length of fore, mid and hind femur was recorded highest in yellowish green morphotypes (3.42±0.03 mm; 3.83±0.03 mm; 3.24±0.04 mm) and it was observed lowest in brownish morphotype (3.38±0.02; 3.78±0.09; 3.17±0.02 mm).

4.1.2.5. Morphometry of larva, pupa and adult of *Helicoverpa armigera* morphotypes on okra at Thrissur in 2014

The incidence of *H. armigera* was noticed on okra during 4th and 5th standard week of 2014 at Thrissur. Similar to the previous season, six larval morphotypes were observed. The larval length, width, weight and width of head capsule varied significantly among morphotypes (Plate 6).

Larval length was recorded highest in yellowish green morphotype (28.00±0.47 mm) and the lowest in greenish morphotype (24.05±0.39 mm). Similarly, larval width was observed highest in yellowish green morphotype (3.45±0.43 mm), whereas lowest larval width was noticed in brownish morphotype (3.25±0.27 mm). Yellowish green morphotypes had the highest larval weight and

width of head capsule (313.91±16.09 mg; 2.25±0.06 mm), whereas greenish morphotypes was recorded the lowest larval weight (237.09±15.71 mg) and width of head capsule (2.19±0.08 mm) (Table 8a).

Male pupal parameters *viz*., length, width and weight varied significantly among morphotypes. Pupa emerged from yellowish green morphotype had the highest pupal length (16.25±0.35 mm), followed by light green (15.90±0.73 mm), whereas brownish morphotype was recorded lowest pupal length (14.48±0.05 mm). Highest pupal width was observed in light green morphotype (3.45±0.49 mm) and the lowest in both greenish and brownish morphotypes (3.11 mm). Pupal weight was recorded highest in yellowish green morphotype (213.90±13.19 mg), followed by light green (196.19±18.61 mg), however lowest pupal weight was noticed in brownish morphotype (165.55±23.05 mg).

Female pupal length and width was recorded highest in yellowish green morphotype (16.32±0.36 mm; 3.41±0.31 mm), whereas lowest in brownish morphotype (14.88±0.51 mm; 3.15±0.24 mm). Similarly, yellowish green morphotype had the highest pupal weight (224.87±16.75 mg), however lowest pupal weight was observed in brown morphotype with dark brown longitudinal lines (175.19±20.74 mg).

Male moths emerged from light green morphotype had the highest length of forewing (15.95±0.79 mm), followed by yellowish green (15.65±6.47 mm), whereas male moths of brownish morphotype recorded lowest length of forewing (14.41±0.66 mm). Width of forewing was observed highest in yellowish green morphotype (7.21±0.24 mm), followed by light green (7.05±0.55 mm) and the lowest in brownish morphotypes (6.08±0.30 mm). Adults of light green with orange spots had highest length of fore, mid and hind femur (3.40±0.03 mm; 3.83±0.06 mm; 3.23±0.03 mm) and lowest length of fore, mid and hind femur observed in brownish (3.35±0.02 mm),

Table 8a. Morphometry of larva and pupa of *Helicoverpa armigera* morphotypes on okra (during 4th and 5th standard week of 2014) at Thrissur

Morphotype	Morphometry of immature stages of *H. armigera*									
	Larva				Pupa					
					Male			Female		
	Length (mm)	Width (mm)	Weight (mg)	Width of head capsule (mm)	Length (mm)	Width (mm)	Weight (mg)	Length (mm)	Width (mm)	Weight (mg)
Light green	26.40±1.39	3.65±0.44	292.85±18.17	2.19±0.06	15.90±0.73	3.45±0.49	196.19±18.61	15.95±0.76	3.45±0.43	197.31±19.88
Light green with orange spots	25.65±0.74	3.35±0.24	269.32±28.60	2.23±0.06	15.70±0.42	3.10±0.56	176.44±9.22	15.75±0.35	3.16±0.25	180.05±8.90
Yellowish green	28.00±0.47	3.45±0.43	313.91±16.09	2.25±0.06	16.25±0.35	3.22±0.34	213.90±13.19	16.32±0.36	3.41±0.31	224.87±16.75
Greenish	24.05±0.39	3.33±0.25	237.09±15.71	2.19±0.08	15.05±0.39	3.11±0.75	168.77±28.05	15.26±0.26	3.27±0.26	188.63±26.22
Brownish	24.16±0.75	3.25±0.27	257.95±24.57	2.21±0.07	14.48±0.50	3.11±0.09	165.55±23.09	14.88±0.51	3.15±0.24	176.19±20.74
Brown with dark brown longitudinal lines	25.62±0.62	3.37±0.25	265.88±16.11	2.26±0.03	15.19±0.81	3.15±0.65	173.36±9.51	15.62±0.25	3.18±0.40	175.62±1.70
Mean	**25.80±1.61**	**3.41±0.25**	**267.21±32.80**	**2.22±0.07**	**15.57±0.75**	**3.13±0.39**	**181.33±28.80**	**15.62±0.63**	**3.28±0.32**	**184.43±28.04**
CD(p=0.05)	1.12	0.45	27.92	0.08	0.67	0.37	25.37	0.64	NS	25.71

Mean of 50 observations

light green with orange spots (3.76±0.03 mm) and brownish morphotypes (3.16±0.02 mm) respectively (Table 8b).

In female moths, highest length of forewing was recorded in light green morphotype (16.05±0.83 mm) and the lowest in brownish morphotypes (14.75±0.93 mm), whereas, width of forewing was observed highest in yellowish green morphotype (7.30±0.25 mm) and the lowest in brownish morphotype (6.16±0.25 mm).

Female moths of yellowish green morphotype had highest length of fore, mid and hind femur (3.42±0.05mm; 3.84±0.03 mm; 3.23±0.01 mm) and the lowest length of fore femur was recorded in both greenish and brownish morphotype (3.37 mm), mid femur in both greenish and brown with dark brown longitudinal lines (3.79 mm) and hind femur in brownish morphotype (3.17±0.05 mm).

4.1.2.6. Morphometry of larva, pupa and adult of *Helicoverpa armigera* morphotypes on chickpea at Thrissur during 2012-13.

Incidence of *H. armigera* on chickpea was observed from 49th standard week of 2012 to 1st standard week of 2013 at Thrissur 1st standard week of 2013. Four larval morphotypes *viz*., green with green longitudinal lines, greenish, green with brown longitudinal lines and brown with brown longitudinal lines were recorded (Plate 7). Larval parameters viz., length, width, weight and width of head capsule varied significantly among morphotypes.

Green with green longitudinal lines had the highest larval length (24.30±0.83 mm), followed by green with brown longitudinal lines (23.55±1.32 mm), brown with brown longitudinal lines (23.32±1.29 mm) and the lowest larval length was recorded in greenish morphotypes (22.75±1.55 mm).Width of larva was observed highest in green with green longitudinal lines (3.26±0.29 mm), subsequently greenish (3.25±0.25 mm), brown with brown longitudinal lines (3.23±0.25 mm) and lowest

Table 8b. Morphometry of adult of *Helicoverpa armigera* morphotypes on okra (during 4th and 5th standard week of 2014) at Thrissur

Morphotype	Morphometry of *H. armigera* adult moth									
	Male					Female				
	Length of forewing (mm)	Width of Forewing (mm)	Length of fore femur (mm)	Length of mid femur (mm)	Length of hind femur (mm)	Length of forewing (mm)	Width of Forewing (mm)	Length of fore femur (mm)	Length of mid femur (mm)	Length of hind femur (mm)
Light green	15.95±0.79	7.05±0.55	3.36±0.01	3.77±0.04	3.18±0.11	16.05±0.83	7.10±0.51	3.40±0.03	3.81±0.04	3.19±0.01
Light green with orange spots	15.03±0.47	6.90±0.45	3.39±0.02	3.76±0.03	3.18±0.01	15.10±0.45	6.95±0.49	3.39±0.06	3.80±0.03	3.19±0.16
Yellowish green	15.65±0.47	7.21±0.24	3.40±0.03	3.83±0.06	3.23±0.03	15.80±0.53	7.30±0.25	3.42±0.05	3.84±0.03	3.23±0.01
Greenish	15.25±0.72	6.77±0.65	3.38±0.02	3.78±0.04	3.19±0.01	15.27±0.56	6.73±0.56	3.37±0.01	3.79±0.05	3.18±0.09
Brownish	14.41±0.66	6.08±0.30	3.35±0.02	3.79±0.04	3.16±0.02	14.75±0.93	6.16±0.25	3.37±0.04	3.80±0.05	3.17±0.05
Brown with dark brown longitudinal lines	15.01±0.56	6.97±0.25	3.36±0.09	3.78±0.09	3.19±0.02	15.21±0.25	7.01±0.25	3.41±0.,03	3.79±0.04	3.19±0.05
Mean	**15.52±0.63**	**6.81±0.54**	**3.39±0.03**	**3.79±0.04**	**3.19±0.02**	**15.42±0.75**	**6.95±0.55**	**3.39±0.02**	**3.80±0.05**	**3.19±0.02**
CD(p=0.05)	0.75	0.60	0.04	0.04	0.06	0.87	0.59	0.06	NS	0.06

Mean of 50 observations

a. Green with green longitudinal lines

b. Greenish

c. Green with brown longitudinal lines

d. Brown with brown longitudinal lines

Plate 7. *Helicoverpa armigera* larval morphotypes on chickpea

larval width was noticed in green with brown longitudinal lines (3.19±0.13 mm) were followed (Table 9a).

Green with green longitudinal lines had the highest larval weight (248.39±26.51 mg), succeeded by brown with brown longitudinal lines (243.29±28.48 mg), green with brown longitudinal lines (242.49±23.25 mg), lowest larval weight was recorded in greenish morphotype (237.01±28.52 mg).Width of head capsule of green with green longitudinal lines was observed highest (2.20±0.02 mm), whereas lowest in greenish morphotypes (2.15±0.06 mm).

Male and female pupal parameters showed non significant variation among morphotypes. Highest male pupal weight was recorded in green with green longitudinal lines (15.22±0.99 mm), followed by brown with brown longitudinal lines (15.15±0.96 mm), greenish (15.13±0.89 mm) and green with brown longitudinal line s (14.68±2.24 mm). Width of male pupa was observed highest in green with green longitudinal line s (3.13±0.30 mm), succeeded by both greenish and brown morphotype with brown longitudinal lines (3.09 mm) and the lowest in greenish morphotype (3.07±0.53 mm). Green with green longitudinal lines had the highest pupal weight (173.53±21.26 mg), followed by greenish morphotypes (170.29±28.06 mg) and it was observed in both green and brown morphotypes with brown longitudinal lines (166 mm).

Female pupal length, width and weight were recorded highest in green colormorph with green longitudinal lines (15.25±0.94 mm; 3.20±0.26 mm; 179.19±20.18 mg), whereas lowest pupal length was observed in green with brown longitudinal lines (14.76±2.22 mm), pupal width in greenish morphotype (3.17±0.25 mm and pupal weight recorded in brown with brown longitudinal lines (170.42±26.59 mg).

Table 9a. Morphometry of larva and pupa of *Helicoverpa armigera* morphotypes on chickpea (from 49th standard week of 2012 to 1st standard week of 2013) at Thrissur

Morphotype	Morphometry of immature stages of *H. armigera*									
	Larva				Pupa					
					Male			Female		
	Length (mm)	Width (mm)	Weight (mg)	Width of head capsule (mm)	Length (mm)	Width (mm)	Weight (mg)	Length (mm)	Width (mm)	Weight (mg)
Green with green longitudinal lines	24.30±0.83	3.26±0.29	248.39±26.51	2.20±0.02	15.22±0.99	3.13±0.30	173.53±21.26	15.25±0.94	3.20±0.26	179.19±20.18
Greenish	22.75±1.55	3.25±0.25	237.01±28.52	2.15±0.06	15.13±0.89	3.07±0.53	170.29±21.06	15.13±0.79	3.17±0.25	176.17±22.09
Green with brown longitudinal lines	23.55±1.32	3.19±0.13	242.49±23.25	2.19±0.08	14.68±2.24	3.09±0.45	166.03±17.51	14.76±2.22	3.19±0.24	172.64±15.61
Brown with brown longitudinal lines	23.32±1.29	3.23±0.25	243.29±28.48	2.19±0.06	15.15±0.96	3.09±0.22	166.22±27.19	15.09±0.77	3.19±0.30	170.42±26.59
Mean	**23.58±1.36**	**3.25±0.27**	**243.60±26.85**	**2.18±0.08**	**15.09±1.30**	**3.10±0.50**	**170.28±21.38**	**15.10±1.25**	**3.19±0.26**	**175.87±20.83**
CD(p=0.05)	1.62	0.07	0.11	0.06	NS	NS	NS	NS	NS	NS

Mean of 50 observations

Length of forewing of adult male moth was observed the highest in green with green longitudinal lines (15.28±0.75 mm), followed by brown morphotype with brown longitudinal lines (15.23±0.93 mm), greenish (15.19±0.78 mm) morphotype and lowest in green with brown longitudinal lines (14.89±0.82 mm) (table 9b). Green with green longitudinal lines had the highest width of forewing (6.88±0.52 mm), followed by greenish (6.74±0.57 mm), green with brown longitudinal lines (6.71±0.42 mm) and lowest in brown with brown longitudinal lines (6.68±0.90 mm).Length of fore, mid and hind femur were recorded highest in green with green longitudinal lines (3.41±0.03 mm; 3.82±0.04 mm; 3.19±0.04 mm) and the lowest length of fore femur was observed in brown with brown longitudinal lines (3.37±0.03 mm), mid femur in both green and brown morphotypes with brown longitudinal lines (3.76 mm) and hind femur in green with brown longitudinal lines (3.15±0.04 mm).

In female moths length and width of fore wing was recorded highest in green with green longitudinal lines (15.68±0.80 mm; 6.98±0.48 mm), whereas lowest length of forewing was noticed in green with brown longitudinal lines (14.98±0.83 mm) and width of forewing in greenish morphotype (6.78±0.55 mm). Highest length of fore, mid and hind femur was observed in adult female moths of green with green longitudinal lines (3.41±0.03 mm; 3.81±0.06 mm; 3.20±0.04 mm) and lowest length of fore femur in greenish morphotype (3.39±0.02 mm) and lowest length of mid and hind femur recorded in green morphotype with brown longitudinal lines (3.77±0.05 mm; 3.18±0.09 mm).

4.1.2.7. Morphometry of larva, pupa and adult of *Helicoverpa armigera* morphotypes on amaranthus at Thrissur in 2013.

Infestation of *H. armigera* was noticed on amaranthus from 19th to 22nd standard week of 2013 at Thrissur. Five larval morphotypes *viz.*, light green, greenish, green with dark longitudinal lines, brown with orange spots and brown with dark dorsal lines were observed (Plate 8). Larval length and width of head capsule

Table 9b. Morphometry of adult of *Helicoverpa armigera* morphotypes on chickpea (from 49th standard week of 2012 to 1st standard week of 2013) at Thrissur

Morphotype	Morphometry of *H. armigera* adult moth									
	Male					Female				
	Length of forewing (mm)	Width of Forewing (mm)	Length of fore femur (mm)	Length of mid femur (mm)	Length of hind femur (mm)	Length of forewing (mm)	Width of Forewing (mm)	Length of fore femur (mm)	Length of mid femur (mm)	Length of hind femur (mm)
Green with green longitudinal lines	15.28±0.75	6.88±0.52	3.41±0.03	3.82±0.04	3.19±0.04	15.68±0.80	6.98±0.48	3.41±0.03	3.81±0.06	3.20±0.04
Greenish	15.19±0.78	6.74±0.57	3.40±0.04	3.79±0.04	3.17±0.05	15.43±1.52	6.78±0.55	3.39±0.02	3.80±0.04	3.19±0.05
Green with brown longitudinal lines	14.89±0.82	6.71±0.42	3.38±0.04	3.76±0.06	3.15±0.04	14.98±0.83	6.92±0.50	3.42±0.05	3.77±0.05	3.18±0.09
Brown with brown longitudinal lines	15.23±0.93	6.68±0.90	3.37±0.03	3.76±0.04	3.17±0.05	15.47±1.07	6.89±0.29	3.40±0.03	3.79±0.06	3.19±0.05
Mean	**15.16±0.70**	**6.75±0.59**	**3.40±0.04**	**3.79±0.05**	**3.17±0.04**	**15.47±0.87**	**6.84±0.87**	**3.40±0.03**	**3.79±0.06**	**3.19±0.05**
CD(p=0.05)	NS	NS	NS	0.06	0.08	NS	0.68	NS	NS	NS

Mean of 50 observations

a. Light green

b. Greenish

c. Green with dark longitudinal lines

d. Brown with orange spots

e. Brown with dark dorsal lines

Plate 8. *Helicoverpa armigera* larval morphotypes on amaranthus

varied significantly among morphotypes whereas, a non significant variation observed with respect to larval width and weight (Table 10a).

Light green morphotype had the highest larval length (23.27±1.05 mm), followed by green with dark longitudinal lines (22.21±0.89 mm), brown with orange spots (22.04±1.23 mm), brown with dark dorsal lines (21.70±0.75 mm) and the lowest larva length was recorded in greenish morphotype (21.50±1.13 mm). Larval width was observed highest in light green morphotype (3.27±0.26 mm), succeeded by green with dark longitudinal lines (3.21±0.28 mm), brown with orange spots (3.19±0.36 mm), greenish (3.18±0.25 mm) and brown with dark dorsal lines (3.08±0.66 mm).

Light green morphotype had the highest larval weight (228.25±36.40 mg), followed by brown with orange spots (227.18±18±33.22 mg), green with dark longitudinal lines (225.87±26.39 mg), greenish (222.63±16.16 mg) and brown with dark dorsal lines (205.98±14.09 mg). Width of head capsule was observed highest in both light green and brown with orange spots (2.21 mm), and it was recorded lowest in brown morphotype with dark dorsal lines (2.18±0.06 mm).

Both male and female pupal parameters showed a non significant variation among morphotypes. Length and width of male pupa was recorded highest in light green morphotype (15.22±0.78 mm; 3.18±0.26 mm), whereas lowest in greenish morphotypes (14.56±0.22 mm; 3.06±0.17 mm). Male pupal weight was observed highest in brown with orange spots (172.57±21.21 mg), followed by both light green and brown with dark dorsal lines (169 mg), green with dark longitudinal lines (165.80±13.20 mg) and lowest pupal weight was noticed in greenish morphotype (163.17±18.78 mg). Female pupal length, width and weight was recorded highest in light green (15.39±0.86 mm; 3.41±0.26 mm; 179.21±22.13 mg), whereas lowest in pupa of greenish morphotype (14.63±3.33 mm; 3.21±0.25 mm; 164.19±18.8 mg).

Table 10a. Morphometry of larva and pupa of *Helicoverpa armigera* morphotypes occurring on amaranthus (during 19th to 22nd standard week of 2013) at Thrissur

Morphometry of immature stages of *H. armigera*										
	Larva				Pupa					
					Male			Female		
Morphotype	**Length (mm)**	**Width (mm)**	**Weight (mg)**	**Width of head capsule (mm)**	**Length (mm)**	**Width (mm)**	**Weight (mg)**	**Length (mm)**	**Width (mm)**	**Weight (mg)**
Light green	23.27±1.05	3.27±0.26	228.25±36.40	2.21±0.01	15.22±0.78	3.18±0.26	169.70±21.98	15.39±0.86	3.41±0.26	179.21±22.13
Greenish	21.50±1.13	3.18±0.25	222.63±16.16	2.20±0.06	14.56±0.22	3.06±0.17	163.17±18.78	14.63±3.33	3.21±0.25	164.19±18.8
Green with dark longitudinal lines	22.21±0.89	3.21±0.26	225.87±26.39	2.20±0.05	14.88±0.45	3.14±0.24	165.80±13.20	14.95±0.37	3.25±0.26	169.73±16.52
Brown with orange spot	22.04±1.23	3.19±0.36	227.18±33.22	2.21±0.08	14.94±0.72	3.16±0.25	172.57±21.21	15.05±0.52	3.29±0.26	170.08±17.59
Brown with dark dorsal lines	21.70±0.75	3.08±0.66	205.98±14.09	2.18±0.06	14.60±0.65	3.10±0.56	169.88±10.72	14.80±0.44	3.28±0.27	171.09±9.78
Mean	**22.12±1.24**	**3.21±0.27**	**223.65±27.36**	**2.20±0.08**	**14.81±0.73**	**3.13±0.22**	**166.30±18.63**	**14.67±1.61**	**3.25±0.15**	**170.75±17.89**
CD(p=0.05)	1.84	NS	NS	0.08	NS	NS	NS	NS	NS	NS

Mean of 50 observations

Adult male moths emerged from light green morphotype had the highest length and width of forewing (15.09±0.83 mm; 6.90±0.49 mm), whereas lowest length of forewing was observed in brown with dark dorsal lines (14.20±0.57 mm) and width in green with dark longitudinal lines (6.30±0.24 mm). Length of fore, mid and hind femur were recorded highest in adult male moths of light green morphotype (3.44±0.04 mm; 3.84±0.02 mm; 3.20±0.02mm), whereas lowest length of fore, mid and hind femur was noticed in brown with dark dorsal lines (3.34±0.04 mm; 3.72±0.05 mm; 3.17±0.06 mm) (Table 10b).

In female moths, length and width of forewing was observed highest in light green morphotype (15.09±0.73 mm; 6.96±0.41 mm), whereas lowest in brown with dark dorsal lines (14.48±0.65 mm; 6.61±0.21 mm). Length of fore, mid and hind femur had highest in adult female moths of light green morphotype (3.44±0.03 mm; 3.88±0.03 mm; 3.21±0.02mm), however lowest length of fore, mid and hind femur were noticed in brown with dark dorsal lines (3.41±0.02 mm; 3.76±0.04 mm; 3.18±0.04 mm).

4.1.3. Morphometry of larva, pupa and adult of *Helicoverpa armigera* reared on tomato fruits and semi synthetic diet

Larval morphotypes of *H. armigera* collected from tomato were brought to laboratory and fed with both tomato fruits and chickpea based semi synthetic diet and morphometric parameters of larva, pupa and adult of morphotypes were studied. Among the morphotypes reared on tomato fruits, both greenish and brown with white lateral lines had the highest larval length (25.70 ±0.75mm), followed by light green with orange spots (25.60±0.35 mm), light green (25.10±1.47 mm), green with dark green dorsal lines (21.10±1.05 mm), brown with orange spots (20.90±0.74 mm) and lowest larval length was observed in green with black lines and spots (19.80±0.57 mm) (Table 11a). among morphotypes reared on semi synthetic diet, green morphotype had the highest larval length (25.50±0.70 mm), followed by light green

Table 10b. Morphometry of adult of *Helicoverpa armigera* morphotypes on amaranthus (during 19th to 22nd standard week of 2013) at Thrissur

Morphotype	Morphometry of *H. armigera* adult moth									
	Male					Female				
	Length of forewing (mm)	Width of Forewing (mm)	Length of fore femur (mm)	Length of mid femur (mm)	Length of hind femur (mm)	Length of forewing (mm)	Width of Forewing (mm)	Length of fore femur (mm)	Length of mid femur (mm)	Length of hind femur (mm)
Light green	15.09±0.83	6.90±0.49	3.44±0.04	3.84±0.02	3.20±0.02	15.09±0.73	6.96±0.41	3.44±0.03	3.88±0.03	3.21±0.02
Greenish	14.37±0.64	6.62±0.52	3.40±0.03	3.83±0.07	3.19±0.05	14.43±0.67	6.81±0.05	3.42±0.03	3.78±0.14	3.19±0.05
Green with dark longitudinal lines	14.50±0.40	6.30±0.24	3.44±0.04	3.79±0.03	3.19±0.07	14.57±0.53	6.89±0.26	3.42±0.06	3.81±0.04	3.20±0.05
Brown with orange spot	14.51±0.55	6.66±0.55	3.34±0.04	3.77±0.07	3.18±0.05	14.77±0.61	6.61±0.41	3.41±0.03	3.80±0.04	3.20±0.03
Brown with dark dorsal lines	14.20±0.57	6.40±0.22	3.35±0.05	3.72±0.05	3.17±0.06	14.48±0.65	6.61±0.21	3.41±0.02	3.76±0.04	3.18±0.04
Mean	**14.67±0.04**	**6.63±0.43**	**3.41±0.05**	**3.80±0.06**	**3.18±0.05**	**14.71±0.67**	**6.69±0.47**	**3.42±0.04**	**3.80±0.07**	**3.19±0.04**
CD(p=0.05)	NS	NS	0.08	NS	0.08	NS	NS	0.06	0.04	0.08

Mean of 50 observations

with orange spots (25.34±1.02 mm), brown with white lateral lines (24.75±1.25 mm), light green (24.61±1.08 mm), green with dark green dorsal lines (20.86±0.90 mm), brown with orange spots (20.82±0.50 mm) and green with black lines and spots (19.77±0.83 mm) (Table 12a).

On tomato, greenish morphotype was recorded the highest larval width (3.48±0.35 mm), whereas lowest in green with black lines and spots (3.12±0.27 mm). However on semi synthetic diet, light green morphotype had the highest larval width (3.61±0.41 mm) and lowest in green with black lines and spots (3.20±0.25 mm).

Greenish morphotype reared on tomato had the highest larva weight (285.72±41.46 mg) and a lowest larval weight of 220.83±14.13 mg in brown with orange spots. Similarly, greenish morphotype reared on semi synthetic diet was recorded the highest larval weight of 280.02±38.14 mg and lowest larva weight recorded in green morphotype with orange spots (219.76±18.13 mg).

Width of larval head capsule of greenish morphotypes reared on tomato was observed highest (2.16±0.07 mm), and the lowest in brown with orange spots (2.13±0.09 mm), whereas on semi synthetic diet, greenish morphotypes had the highest width of larval head capsule (2.15±0.10 mm) and lowest in green with black lines and spots (2.10±0.10 mm).

Male pupa of greenish morphotype was recorded highest pupal length (15.94±0.65 mm) and the lowest in brown with orange spots (14.13±0.27 mm), whereas on semi synthetic diet, pupal length was observed highest in greenish morphotype (15.73±0.35 mm) and lowest in light green morphotype (14.62±0.89 mm). Greenish morphotype reared on both tomato and semi synthetic diet had the highest pupal width of 3.31±0.27 mm and 3.34±0.03 mm respectively. Green with black lines and spots (3.14±0.15 mm) reared on tomato and light green morphotype

Table 11a. Morphometry of larva and pupa of *Helicoverpa armigera* morphotypes reared on tomato fruits

Morphometry of immature stages of *H. armigera*										
Morphotype	Larva				Pupa					
					Male			Female		
	Length (mm)	Width (mm)	Weight (mg)	Width of head capsule (mm)	Length (mm)	Width (mm)	Weight (mg)	Length (mm)	Width (mm)	Weight (mg)
Light green	25.10±1.47	3.42±0.50	250.30±51.67	2.14±0.05	14.81±0.83	3.31±0.27	198.29±29.62	14.82±0.83	3.41±0.22	204.78±35.06
Light green with orange spots	25.60±0.38	3.48±0.35	277.47±14.29	2.15±0.06	15.82±0.75	3.20±0.22	204.34±9.21	15.85±0.35	3.21±0.27	210.27±36.97
Greenish	25.70±0.75	3.51±0.27	285.72±41.14	2.15±0.07	15.94±0.65	3.31±0.27	265.54±30.98	15.95±0.65	3.43±0.27	235.26±28.75
Green with dark green dorsal lines	21.10±1.47	3.40±0.22	207.37±5.37	2.15±0.01	14.83±0.41	3.28±0.27	192.46±6.07	14.20±0.57	3.32±0.27	229.68±10.09
Green with black lines and spots	19.80±0.57	3.21±0.27	221.36±22.87	2.14±0.12	14.91±0.22	3.14±0.15	158.04±41.77	15.30±0.67	3.12±0.17	164.35±38.62
Brown with orange spots	20.90±0.89	3.41±0.35	220.83±14.13	2.13±0.09	14.13±0.27	3.20±0.10	155.71±7.92	15.40±0.35	3.25±0.28	159.20±6.93
Brown with white lateral lines	25.70±0.89	3.46±0.40	250.20±10.63	2.15±0.02	15.52±0.40	3.24±0.25	200.89±28.58	15.62±0.47	3.41±0.15	210.28±20.97
Mean	**23.27±2.60**	**3.45±0.55**	**251.28±43.54**	**2.14±0.10**	**15.10±0.79**	**3.22±0.25**	**194.08±35.94**	**15.22±0.81**	**3.30±0.24**	**198.25±35.72**
CD (p=0.05)	2.14	NS	52.78	0.18	1.03	0.40	46.92	1.78	NS	46.36

Mean of 25 observations

Table 12a. Morphometry of larva and pupa of *Helicoverpa armigera* morphotypes reared on semi synthetic diet

Morphometry of immature stages of *H. armigera*										
Morphotype	Larva				Pupa					
					Male			Female		
	Length (mm)	Width (mm)	Weight (mg)	Width of head capsule (mm)	Length (mm)	Width (mm)	Weight (mg)	Length (mm)	Width (mm)	Weight (mg)
Light green	24.61±1.08	3.41±0.41	246.05±47.74	2.14±0.05	14.62±0.89	3.21±0.27	197.97±28.93	14.73±0.83	3.24±0.27	201.34±27.41
Light green with orange spots	25.34±1.02	3.42±0.20	275.26±18.78	2.10±0.06	15.62±0.82	3.02±0.04	202.43±12.11	15.63±0.83	3.10±0.25	209.82±5.85
Greenish	25.51±0.70	3.61±0.41	285.72±41.14	2.17±0.10	15.73±0.35	3.34±0.03	223.70±32.98	15.62±0.79	3.32±0.27	229.44±29.05
Green with dark green dorsal lines	20.86±0.90	3.42±0.22	206.17±5.59	2.15±0.09	13.90±0.65	3.02±0.22	190.83±5.20	14.19±0.98	3.12±0.25	189.83±9.22
Green with black lines and spots	19.77±0.83	3.21±0.27	219.76±18.13	2.10±0.10	14.83±0.27	3.15±0.29	154.01±5.20	15.18±0.50	3.10±0.28	164.34±38.62
Brown with orange spots	20.82±0.83	3.22±0.35	220.82±14.13	2.12±0.06	14.75±0.44	3.25±0.22	150.99±10.36	14.59±0.15	3.24±0.28	157.36±7.99
Brown with white lateral lines	24.75±1.25	3.37±0.47	268.24±21.81	2.15±0.07	15.25±0.63	3.34±0.25	220.32±40.86	15.12±0.85	3.30±0.25	216.42±33.62
Mean	**23.01±2.52**	**3.36±0.33**	**245.36±38.84**	**2.10±0.10**	**14.92±0.85**	**3.17±0.23**	**190.62±36.53**	**14.95±0.92**	**3.20±0.25**	**194.89±33.70**
CD (p=0.05)	1.77	NS	51.42	0.15	1.23	1.16	49.55	NS	NS	45.44

Mean of 25 observations

with orange spots (3.02±0.04 mm) reared on semi synthetic diet was recorded lowest pupal width.

Greenish morphotype reared on both tomato and semi synthetic diet had the highest male pupal weight of 265.54±30.98 mg and 223.70±32.98 mg respectively, whereas lowest pupal weight was recorded in brown morphotype with orange spots reared on both tomato fruits (155.71±7.92 mg) and semi synthetic diet (150.99±10.36 mg).

Female pupal length, width and weight were observed highest in greenish morphotype reared on both tomato fruits (15.95±0.65 mm; 3.43±0.27 mm; 235.26±28.75 mm) and semi synthetic diet (15.62±0.79 mm; 3.32±0.27mm; 229.44±29.05 mm), whereas morphotypes reared on both tomato and semi synthetic diet *viz*., green with dark green dorsal lines, green with black lines and spots, brown with orange spots was recorded the lowest pupal length (14.20±0.57 mm; 14.19±0.98 mm), width (3.12±0.17 mm; 3.10±0.28 mm) and weight (159.20±6.93; 157.36±7.99 mm).

Adult male moths emerged from greenish morphotype reared on both tomato and semi synthetic diet was recorded the highest length and width of forewing, 15.81±0.27mm; 7.21±0.15 mm and 15.60±070 mm; 7.11±0.41 mm respectively (Table 11b; table 12b). The lowest length of forewing was recorded in brown morphotype with orange spots reared on both tomato fruits (14.31±0.44 mm) and semi synthetic diet (14.20±0.35 mm) and lowest width of forewing was observed in green morphotype with black lines reared on tomato (6.21±0.35 mm) and green with dark green dorsal lines reared on semi synthetic diet (6.04±0.44 mm).

Length of fore, mid and hind femur were observed highest in male moths emerged from greenish morphotype reared on both tomato fruits (3.19±0.03 mm; 3.81±0.02 mm; 3.11±0.03 mm) and semi synthetic diet (3.18±0.03 mm; 3.79±0.05

Table 11b. Morphometry of adult of *Helicoverpa armigera* morphotypes reared on tomato fruits

Morphotype	Morphometry of *H. armigera* adult moth									
	Male					Female				
	Length of forewing (mm)	Width of Forewing (mm)	Length of fore femur (mm)	Length of mid femur (mm)	Length of hind femur (mm)	Length of forewing (mm)	Width of Forewing (mm)	Length of fore femur (mm)	Length of mid femur (mm)	Length of hind femur (mm)
Light green	14.41±1.54	6.52±0.70	3.15±0.05	3.77±0.04	3.09±0.03	14.42±0.54	6.52±0.70	3.16±0.07	3.79±0.04	3.10±0.03
Light green with orange spots	15.70±0.57	7.01±0.35	3.18±0.03	3.80±0.04	3.10±0.04	15.90±0.54	7.03±0.50	3.19±0.02	3.80±0.03	3.10±0.03
Greenish	15.81±0.27	7.21±0.15	3.19±0.03	3.81±0.02	3.11±0.03	15.83±0.41	7.25±0.44	3.20±0.02	3.84±0.06	3.12±0.04
Green with dark green dorsal lines	14.91±0.70	6.28±0.27	3.17±0.02	3.80±0.04	3.09±0.03	15.60±0.89	6.30±0.27	3.18±0.02	3.80±0.03	3.10±0.03
Green with black lines and spots	14.52±0.41	6.21±0.35	3.16±0.07	3.77±0.03	3.08±0.05	15.20±0.57	6.42±0.41	3.16±0.43	3.79±0.02	3.09±0.04
Brown with orange spots	14.31±0.44	6.40±0.35	3.15±0.07	3.76±0.06	3.08±0.08	14.60±0.65	6.61±0.22	3.16±0.01	3.80±0.05	3.09±0.08
Brown with white lateral lines	15.02±0.25	6.51±0.25	3.16±0.03	3.77±0.04	3.09±0.03	15.75±0.28	6.75±0.28	3.17±0.02	3.81±0.05	3.10±0.03
Mean	**15.16±0.74**	**6.76±0.53**	**3.16±0.05**	**3.78±0.05**	**3.09±0.04**	**15.35±0.76**	**6.67±0.49**	**3.17±0.05**	**3.78±0.05**	**3.10±0.04**
CD (p=0.05)	0.90	0.67	NS	0.08	NS	0.77	NS	NS	0.08	NS

Mean of 25 observations

Table 12b. Morphometry of adult of *Helicoverpa armigera* morphotypes reared on semi synthetic diet

Morphotype	Morphometry of *H. armigera* adult moth									
	Male					Female				
	Length of forewing (mm)	Width of Forewing (mm)	Length of fore femur (mm)	Length of mid femur (mm)	Length of hind femur (mm)	Length of forewing (mm)	Width of Forewing (mm)	Length of fore femur (mm)	Length of mid femur (mm)	Length of hind femur (mm)
Light green	14.11±0.35	6.34±0.59	3.13±0.06	3.77±0.09	3.07±0.04	14.43±0.54	6.50±0.70	3.14±0.08	3.75±0.06	3.08±0.05
Light green with orange spots	15.50±0.25	6.92±0.49	3.16±0.03	3.72±0.05	3.08±0.03	14.91±0.49	6.69±0.61	3.16±0.05	3.75±0.04	3.09±0.02
Greenish	15.60±0.70	7.11±0.41	3.18±0.03	3.79±0.05	3.09±0.02	15.92±0.89	7.17±0.41	3.18±0.02	3.83±0.06	3.10±0.04
Green with dark green dorsal lines	15.20±0.23	6.04±0.44	3.16±0.06	3.77±0.05	3.09±0.06	15.67±0.65	6.70±0.45	3.17±0.05	3.74±0.04	3.10±0.03
Green with black lines and spots	14.80±0.25	6.07±0.42	3.15±0.07	3.72±0.04	3.08±0.07	15.28±0.57	6.58±0.61	3.16±0.05	3.78±0.05	3.09±0.03
Brown with orange spots	14.20±0.35	6.43±0.77	3.14±0.06	3.66±0.01	3.07±0.06	14.77±0.67	6.20±0.75	3.14±0.05	3.74±0.03	3.08±0.02
Brown with white lateral lines	15.02±0.50	6.62±0.57	3.16±0.05	3.74±0.07	3.09±0.04	14.70±0.75	6.75±0.28	3.16±0.03	3.75±0.01	3.10±0.02
Mean	**14.91±0.73**	**6.47±0.58**	**3.15±0.06**	**3.74±0.08**	**3.08±0.04**	**15.02±0.81**	**6.57±0.61**	**3.16±0.05**	**3.75±0.07**	**3.09±0.04**
CD (p=0.05)	0.95	0.92	NS	NS	NS	1.23	NS	NS	NS	NS

Mean of 25 observations

mm; 3.08±0.03 mm). Male moths of brown with orange spot reared on tomato and semi synthetic diet had the lowest length of fore (3.15±0.07 mm; 3.14±0.06 mm), mid (3.76±0.06 mm; 3.66±0.01 mm) and hind (3.08±0.08 mm; 3.07±0.06 mm) femur.

Female moths emerged from greenish morphotype reared on both tomato fruits and semi synthetic diet had the highest length (15.90±0.54 mm; 15.92±0.89 mm) and width (7.25±0.44 mm; 7.17±0.41 mm) of fore wing. Brown morphotype with orange spots recorded the lowest length (14.60±0.65 mm; 14.20±0.35 mm) and width (6.30±0.27 mm; 6.20±0.75 mm) of fore wing.

Highest length of fore, mid and hind femur were observed in female moths emerged from greenish morphotype reared on both tomato fruits (3.20±0.02 mm; 3.84±0.03 mm; 3.12±0.04 mm) and semi synthetic diet (3.18±0.02 mm; 3.83±0.06 mm; 3.10±0.03 mm). Male moths of brown with orange spots reared on tomato and semi synthetic diet had the lowest length of fore (3.16±0.01 mm; 3.14±0.05 mm), mid (3.80±0.05 mm; 3.74±0.03 mm) and hind (3.09±0.08 mm; 3.08±0.02 mm) femur.

4.1.4. Frequency (%) of larval morphotypes in population of *Helicoverpa armigera* on different crops

4.1.4.1. Frequency (%) of larval morphotypes in population of *Helicoverpa armigera* on tomato in 2012

Incidence of *H. armigera* in tomato was observed in Palakkad from 33rd to 41st standard week of 2012. Altogether seven different morphotypes were recorded. During 33rd standard week, larvae with both greenish and green with black line and spots were dominant (28.5%), followed by light green**,** light green with orange spots and green with dark green dorsal lines (14.3%), whereas brown morphotypes were not observed during 33rd standard week. Both greenish and green with black lines were dominated during 35th standard week (26.3%), followed by light green (15.8%), light green with orange spots (10.5%), both green with dark green dorsal lines and

brown with orange spots (8.8%) and brown with white lateral lines (3.60%) (Table 13).

Green morphotype with black lines and spots were dominant (41.2%) during 37th standard week, followed by greenish (29.4%), both light green and brown with orange spots (8.80%), both light green with orange spots and green with dark green dorsal lines (5.90%), whereas no brown morphotype with white lateral lines were observed during that period. On 39th standard week, light green with orange spots were dominant (33.3%), followed by green with dark green dorsal lines (21.7%), greenish (20%), light green (8.30%), both green with black lines and spots and brown with white lateral lines (6.70%) and brown with orange spots (3.30%).

Greenish morphotypes were dominant (40%) during 41st standard week, subsequently light green with orange spots (20%), green with dark green dorsal lines (12%), light green (8%) were observed. However, brown morphotypes with orange spots and white lateral lines were not recorded during the period of observation.

In tomato growing area of Palakkad, greenish morphotype were dominant (28.8%) during the period from 33rd to 41st standard week of 2012, followed by green with black lines and spots (24.5%), light green with orange spots (16.8%), green with dark green dorsal lines (12.5%), light green (11.4%), brown with orange spots (4.1%), and brown with white lateral lines (2.11%).

In Thrissur, the incidence of *H. armigera* was noticed on tomato from 44th to 47th standard week of 2012 and altogether six different morphotype were observed. Greenish morphotype were dominant (43.8%) during 44th standard week, followed by light green (25%), both light green with orange spots and green with dark green dorsal lines (12.5%) and green with black lines and spots (6.2%). Brown morphotypes were not observed during 44th standard week (Table 13).

Table 13. Frequency (%) of larval morphotypes in population of *Helicoverpa armigera* on tomato in 2012

Location	Collection period	Light green	Light green with orange spots	Greenish	Green with dark green dorsal lines	Green with black lines and spots	Brown with orange spots	Brown with white lateral lines
Palakkad	33rd SW of 2012	14.3 (0.39)	14.3 (0.39)	28.5 (0.56)	14.3 (0.39)	28.5 (0.56)	0.00 (0.08)	0.00 (0.08)
	35th SW of 2012	15.8 (0.41)	10.5 (0.33)	26.3 (0.53)	8.80 (0.30)	26.3 (0.54)	8.70 (0.30)	3.60 (0.19)
	37th SW of 2012	8.80 (0.31)	5.90 (0.25)	29.4 (0.57)	5.90 (0.25)	41.2 (0.70)	8.80 (0.30)	0.00 (0.08)
	39th SW of 2012	8.30 (0.29)	33.3 (0.62)	20.0 (0.46)	21.7 (0.48)	6.70 (0.26)	3.30 (0.18)	6.70 (0.26)
	41st SW of 2012	8.00 (0.29)	20.0 (0.46)	40.0 (0.68)	12.0 (0.35)	20.0 (0.46)	0.00 (0.08)	0.00 (0.08)
	Mean	**11.04 (0.33)[b]**	**16.8 (0.40)[ab]**	**28.8 (0.53)[a]**	**12.5 (0.35)[b]**	**24.5 (0.49)[ab]**	**4.16 (0.20)[c]**	**2.11 (0.14)[c]**
Thrissur	44th SW of 2012	25.0 (0.52)	12.5 (0.36)	43.8 (0.72)	12.5 (0.36)	6.20 (0.25)	0.00 (0.08)	0.00 (0.08)
	45th SW of 2012	26.3 (0.54)	2.70 (0.16)	36.7 (0.65)	5.30 (0.23)	26.3 (0.54)	0.17 (2.70)	0.00 (0.08)
	46th SW of 2012	28.6 (0.56)	21.4 (0.48)	28.6 (0.56)	14.3 (0.39)	7.10 (0.27)	0.00 (0.08)	0.00 (0.08)
	47th SW of 2012	17.6 (0.43)	5.90 (0.25)	58.8 (0.87)	11.8 (0.35)	5.90 (0.25)	0.00 (0.08)	0.00 (0.08)
	Mean	**24.2 (0.51)[b]**	**10.5 (0.32)[c]**	**42.3 (0.71)[a]**	**10.9 (0.33)[c]**	**11.3 (0.34)[c]**	**0.76 (0.09)[d]**	0.00 (0.08)[d]

Mean of 50 observations, Values in the parenthesis are arc sin transformed values, SW- Standard week

Figures followed by same letter in the column do not differ significantly by DMRT (P=0.05)

During 45th standard week, greenish morphotype were highest (36.7%), and other morphotypes *viz*., light green and green with black lines and spots (26.2%), green with dark green dorsal lines (14.3%), both light green with orange spots and brown with orange spots (2.70%) were also observed. However, during 46th standard week greenish morphotype were dominant (28.6%), followed by light green with orange spots (21.4%), green with dark green dorsal lines (14.3%) and green with black lines and spots (7.10%)

During 47th standard week greenish morphotypes were dominant (58.8%), followed by light green (17.8%), green with dark green dorsal lines (11.8%) and both light green with orange spots and green with black lines and spots (5.49%).

In Thrissur, greenish morphotype were dominant (42.3%) during the period of study, followed by light green (24.2%), green with black lines and spots (11.9%), green with dark green dorsal lines (10.9%), light green with orange spots (1.5%) and brown with orange spots (0.76%) were followed.

4.1.4.2. Frequency (%) of larval morphotypes in population of *Helicoverpa armigera* on okra during 2012 and 2013

On okra, the incidence of *H. armigera* was observed from 41st to 47th standard week of 2012 and 7th to 10th standard week of 2013 in Thrissur. Altogether six different larval morphotypes were recorded. During 44th standard week, light green with orange spot morphotypes were dominant (29.4%). It was followed by brown with dark brown longitudinal lines (23.5%), greenish (17.6%), both light green and brownish morphotype (11.8%) and yellowish green (5.90%). Whereas, on 45th standard week more yellowish green morphotypes were observed (36.4%), followed by light green (27.2%), brownish (18.2%), and light green with orange spots (9.10%) (Table 14).

Table 14. Frequency (%) of larval morphotypes in population of *Helicoverpa armigera* on okra during 2012 and 2013

Location	Collection period	Light green	Light green with orange spots	Yellowish green	Greenish	Brownish	Brown with dark brown longitudinal lines
Thrissur	44th SW of 2012	11.8 (0.35)	29.4 (0.57)	5.90 (0.24)	17.6 (0.43)	0.35 (11.8)	23.50 (0.51)
	45th SW of 2012	27.2 (0.55)	9.10 (0.31)	36.4 (0.65)	9.10 (0.31)	0.44 (18.2)	0.00 (0.007)
	46th SW of 2012	27.8 (0.55)	5.60 (0.24)	33.3 (0.61)	22.1 (0.49)	0.34 (11.1)	0.00 (0.007)
	47th SW of 2012	36.4 (0.65)	9.10 (0.31)	54.5 (0.83)	0.00 (0.007)	0.007 (0.00)	0.00 (0.007)
	7th SW of 2013	41.7 (0.70)	0.00 (0.007)	16.6 (0.42)	41.7 (0.70)	0.007 (0.00)	0.00 (0.007)
	8th SW of 2013	27.3 (0.55)	0.00 (0.007)	36.3 (0.65)	27.3 (0.55)	0.52 (25.0)	0.00 (0.007)
	9th SW of 2013	37.5 (0.66)	0.00 (0.007)	25.0 (0.52)	12.5 (0.36)	0.52 (25.0)	0.00 (0.007)
	10th SWof 2013	28.6 (0.56)	0.00 (0.007)	14.3 (0.39)	35.7 (0.64)	0.48 (21.4)	0.00 (0.007)
	Mean	**29.2 (0.57)[a]**	**6.50 (0.25)[cd]**	**27.3 (0.54)[ab]**	**20.3 (0.46)[ab]**	**13.8 (0.38)[bc]**	**2.89 (0.17)[d]**

Mean of 50 observations, Values in the parenthesis are arc sin transformed values
Figures followed by same letter in the column do not differ significantly by DMRT (P=0.05)

Table 15. Frequency (%) of larval morphotypes in population of *Helicoverpa armigera* on chickpea in 2012

Location	Collection period	Green with green longitudinal lines	Greenish	Green with brown longitudinal lines	Brown with brown longitudinal lines
Thrissur	49th SW of 2012	40.0 (0.68)	20.0 (0.46)	24.0 (0.51)	16.0 (0.41)
	50th SW of 2012	35.3 (0.64)	11.8 (0.35)	32.3 (0.60)	20.6 (0.47)
	51st SW of 2012	33.3 (0.61)	39.4 (0.68)	12.1 (0.36)	15.2 (0.40)
	52nd SW of 2012	42.5 (0.71)	30.0 (0.58)	20.0 (0.46)	7.50 (0.27)
	1st SW of 2013	58.9 (0.87)	32.5 (0.61)	17.6 (0.43)	0.00 (0.01)
	Mean	**41.3 (0.69)[a]**	**26.3 (0.53)[ab]**	**20.8 (0.47)[bc]**	**11.6 (0.35)[c]**

Mean of 50 observations, Values in the parenthesis are arc sin transformed values
Figures followed by same letter in the column do not differ significantly by DMRT (P=0.05)

Yellowish green morphotypes dominated during 46th (33%) and 47th (54%) standard week, followed by light green (27.8%; 36.4%). On 46th standard week the population of morphotypes *viz.*, greenish, brownish and light green with orange spots observed were 22.1 per cent, 11.1 per cent and 5.6 per cent respectively, whereas on 47th standard week, other morphotypes *viz.*, light green (36.4%) and light green with orange spots (9.1%) were also recorded.

On 7th standard week of 2013, both light green and greenish morphotypes were dominant (41.7%), followed by yellowish green (16.61%), whereas on 8th standard week yellowish green were dominant (36.3%), followed by both light green and greenish (27.3%) and brownish morphotype (25%) (Table 11). However, on 9th standard week, light green morphotype recorded highest (37.5%), followed by both yellowish green and brownish (25%) and greenish morphotype (12.5%). On 10th standard week, greenish morphotypes were dominant (35.7%), other morphotypes *viz.*, light green (28.6%), brownish (21.4%), yellowish green (14.3%) morphotypes were also recorded.

By considering the mean population of morphotypes occurred on okra, light green morphotype were dominant (29.2%), followed by yellowish green (27.3%), greenish (20.3%), brownish (13.8%), light green with orange spots (6.5%) and brown with dark brown longitudinal lines (2.89%).

4.1.4.3. Frequency (%) of larval morphotypes in population of *Helicoverpa armigera* on chickpea during 2012

In chickpea four different morphotypes of *H. armigera* were observed from 49th standard week of 2012 to 1st standard week of 2013. Green with green dorsal lines were dominant (40%), followed by green with brown longitudinal lines (24%), greenish (20%) and brown with brown longitudinal lines (16%). During 50th standard week, larval morphotypes recorded were green with green longitudinal lines (35.3%),

green with brown longitudinal lines (32.3%), brown with brown longitudinal lines (20.6%) and greenish morphotypes (11.8%) (Table 15).

Greenish morphotypes were dominant (39.4%) in the population of *H. armigera* collected from chickpea on 51st standard week, followed by green with green dorsal lines (33.3%), brown with brown dorsal lines (15.2%) and green with brown dorsal lines (12.1%).

However, on 51st standard week of 2012 and 1st standard week of 2013 greenish morphotype were dominant (42.5%; 58.9%), followed by greenish (30%; 32.5%), green with brown dorsal lines (20%; 17.1%) and brown with brown dorsal lines during 52nd standard week (7.5%).

In chickpea, green morphotype with green dorsal line were dominant (41.3%) during the period of observation, followed by greenish (26.3%), green with brown dorsal lines (20.8%) and brown with brown dorsal lines (11.6%)

4.1.4.4. Frequency (%) of larval morphotypes in population of *Helicoverpa armigera* on amaranthus in 2013

Incidence of *H. armigera* on amaranthus at Thrissur was observed from19th to 22nd standard week of 2013. Five morphotypes were recorded during that period. On 19th standard week, light green, yellowish green with dark dorsal lines and brown with orange spots (28.6% each) and greenish morphotypes (14.2%) were recorded. On 20th standard week, light green morphotype were dominant (41.7%), followed by brown with orange spots (25%), brown with dark dorsal lines (16.7%) and both greenish and yellowish green morphotypes (8.3%) (Table 16).

Brown with dark dorsal lines dominated (37.5%) during 21st standard week, subsequently morphotype *viz.,* yellowish green with dark longitudinal lines (25%), light green, greenish and brown with orange spots (12.5% each) were followed.

Table 16. Frequency (%) of larval morphotypes in population of *Helicoverpa armigera* on amaranthus in 2013

Location	Collection period	Light green	Greenish	Yellowish Green with dark longitudinal lines	Brown with orange spot	Brown with dark longitudinal lines
Thrissur	19th SW of 2013	28.6 (0.56)	14.2 (0.39)	28.6 (0.56)	28.6 (0.56)	0.00 (0.01)
	20th SW of 2013	41.7 (0.70)	8.30 (0.29)	8.30 (0.29)	25.0 (0.52)	16.7 (0.41)
	21st SW of 2013	12.5 (0.36)	12.5 (0.36)	25.0 (0.52)	12.5 (0.36)	37.5 (0.65)
	22nd SW of 2013	0.00 (0.10)	33.3 (0.62)	50.0 (0.78)	16.7 (0.42)	0.00 (0.01)
	Mean	**20.7 (0.47)[a]**	**17.1 (0.42)[a]**	**27.9 (0.55)[a]**	**20.7 (0.47)[a]**	**13.6 (0.37)[a]**

Mean of 50 observations, Values in the parenthesis are arc sin transformed values, SW- Standard week
Figures followed by same letter in the column do not differ significantly by DMRT (P=0.05)

Table 17. Frequency (%) of larval morphotypes in population of *Helicoverpa armigera* on tomato in 2013

Location	Collection period	light green	light green with orange spots	Greenish	Green with dark green dorsal lines	Green with black lines and spots	Brown with orange spots	Brown with white lateral lines
Palakkad	35th SW of 2013	23.8 (0.51)	9.50 (0.31)	33.3 (0.61)	14.3 (0.39)	19.1 (0.45)	0.00 (0.01)	0.00 (0.01)
	37th SW of 2013	22.2 (0.49)	13.3 (0.37)	26.7 (0.54)	11.1 (0.33)	15.6 (0.40)	4.40 (0.21)	6.70 (0.26)
	39th SW of 2013	23.1 (0.50)	12.8 (0.37)	25.6 (0.53)	10.3 (0.33)	15.4 (0.40)	5.10 (0.22)	7.70 (0.28)
	41st SW of 2013	19.2 (0.45)	15.4 (0.40)	34.6 (0.63)	11.5 (0.35)	7.70 (0.28)	0.00 (0.01)	0.00 (0.01)
	Mean	**22.7 (0.49)[ab]**	**13.1 (0.37)[bc]**	**30.9 (0.58)[a]**	**12.1 (0.35)[c]**	**14.9 (0.39)[bc]**	**2.42 (0.15)[d]**	**3.70 (0.19)[d]**

Mean of 50 observations, Values in the parenthesis are arc sin transformed values, SW- Standard week
Figures followed by same letter in the column do not differ significantly by DMRT (P=0.05)

Yellowish green with dark longitudinal lines were dominant (50%) during 22nd standard week, followed by greenish (33.3%) and brown with orange spots (16.7%).

Yellowish green morphotype with dark longitudinal lines were dominant (27.9%) in amaranthus during the period of study, followed by both light green and brown with orange spots (20.7%), greenish (17.1%) and brown with dark longitudinal lines (13.6%).

4.1.4.5. Frequency (%) of larval morphotypes in population of *Helicoverpa armigera* on tomato in 2013

During 2013, the incidence of *H. armigera* was observed on tomato in Palakkad between 35th and 41st standard weeks and altogether seven larval morphotypes were recorded. On 35th standard week, greenish morphotype was dominant (33.3%) followed by light green (23.8%), green with black lines and spots (19.1%), green with dark green dorsal lines (14.3%) and light green with orange spots (9.50%) (Table 17). On 37th standard week greenish morphotype was recorded highest (26.7%) and other morphotypes *viz.,* light green (22.2%), light green with orange spots (13.3%), green with dark green dorsal lines (11.1%), brown with white lateral lines (6.70%) and brown with orange spots (4.40%) were also recorded.

Greenish morphotype was dominant (25.6%) on 39th standard week, followed by light green (23.1%), green with black lines and spots (15.4%), light green with orange spots (12.8%), green with dark green dorsal lines (10.3%), brown with white lateral lines (7.70%) and brown with orange spots (5.10%). On 41st standard week, greenish morphotype recorded highest (34.6%), followed by light green (19.2%), light green with orange spots (15.4%), green with dark green dorsal lines (11.5%) and green with black lines and spots (7.70%).

During the period of study, greenish morphotype was dominant (30.9%) one, followed by light green (22.7%), green with black line and spots (14.9%), light green

with orange spots (13.1%), green with dark green dorsal lines (12.1%), brown with white lateral lines (3.7%) and brown with orange spots (2.42%).

4.1.4.6. Frequency (%) of larval morphotypes in population of *Helicoverpa armigera* on okra in 2014

The incidence of *H. armigera* was noticed on okra during 4th and 5th standard week of 2014 at Thrissur. On 4th standard week, yellowish green morphotype was dominant (28.6%), followed by light green (23.8%), brownish (19.1%), light green with orange spots (14.3%), greenish (9.50%), brown with dark brown longitudinal lines were followed. Similarly on 5th standard week yellowish green morphotype dominated (34.8%) and light green (26.1%), greenish (17.4%), light green with orange spots (13%), and brownish morphotype (8.70%) were also recorded (Table 18).

On okra the mean population of yellowish green morphotype was highest (31.9%), followed by light green (24.9%), light green with orange spots (13.6%), greenish (13.4%), brownish (3.80%) and brown with dark brown longitudinal lines (2.40%).

4.1.5. Relative frequency (%) of larva with pigmentation on lateral bands

Pigmentation on lateral bands of *H. armigera* larvae collected from different crops was observed and it varied significantly among different morphotypes. During 2012, 39.9 per cent of *H. armigera* larvae collected from tomato in Palakkad possessed continuous pigmentation on lateral bands, whereas 29.3 per cent of larvae with discontinuous pigmentation on lateral bands and 30.8 per cent of larve without pigmentation on lateral bands (Table 19a). However, during 2013, 53.7 per cent of larvae collected from tomato in Palakkad possessed continuous pigmentation on lateral bands, 25.3 per cent of larve with discontinuous pigmentation on lateral bands and no pigmentation observed in 21 per cent of collected larvae (Table 19c).

Table 18. Frequency (%) of larval morphotypes in population of *Helicoverpa armigera* on okra in 2014

Location	Collection period	Light green	Light green with orange spots	Yellowish green	Greenish	Brownish	Brown with dark brown longitudinal lines
Thrissur	4th SW of 2014	23.8 (0.51)	14.3 (0.39)	28.6 (0.56)	9.50 (0.31)	19.1 (0.45)	4.70 (0.22)
	5th SW of 2014	26.1 (0.54)	13.0 (0.37)	34.8 (0.63)	17.4 (0.43)	8.70 (0.30)	0.00 (0.01)
	Mean	**24.9 (0.52)[a]**	**13.6 (0.37)[a]**	**31.9 (0.60)[a]**	**13.4 (0.37)[a]**	**3.80 (0.38)[a]**	**2.40 (0.15)[b]**

Mean of 50 observations, Values in the parenthesis are arc sin transformed values, SW- Standard week
Figures followed by same letter in the column do not differ significantly by DMRT (P=0.05)

Table 19a. Relative frequency (%) of larvae with pigmentation on lateral bands in *Helicoverpa armigera* on tomato in 2012

Crop	Location	Collection period	Pigmentation on lateral bands		
			Continuous	Discontinuous	No pigmentation
Tomato	Palakkad	33rd SW of 2012	42.8 (0.71)	28.6 (0.56)	28.6 (0.56)
		35th SW of 2012	42.1 (0.71)	19.3 (0.45)	38.6 (0.67)
		37th SW of 2012	38.2 (0.67)	11.8 (0.35)	50.0 (0.78)
		39th SW of 2012	28.3 (0.56)	55.0 (0.84)	16.7 (0.42)
		41st SW of 2012	48.0 (0.77)	32.0 (0.60)	20.0 (0.46)
		Mean	**39.9 (0.68)^a**	**29.3 (0.57)^b**	**30.8 (0.58)^b**
	Thrissur	44th SW of 2012	68.8 (0.98)	25.0 (0.52)	6.20 (0.25)
		45th SW of 2012	63.0 (0.92)	8.00 (0.29)	29.0 (0.57)
		46th SW of 2012	57.2 (0.86)	35.7 (0.64)	7.10 (0.27)
		47th SW of 2012	76.4 (1.06)	17.7 (0.43)	15.9 (0.25)
		Mean	**66.3 (0.95)^a**	**21.6 (0.48)^b**	**12.1 (0.35)^b**

Mean of 50 observations, Values in the parenthesis are arc sin transformed values, SW- Standard week
Figures followed by same letter in the column do not differ significantly by DMRT (P=0.05)

Table 19b. Relative frequency (%) of larvae with pigmentation on lateral bands in *Helicoverpa armigera* on okra and chickpea during 2012 and 2013

Crop	Location	Collection period	Pigmentation on lateral bands		
			Continuous	Discontinuous	No pigmentation
Okra	Thrissur	44th SW of 2012	5.90 (0.24)	35.3 (0.59)	58.8 (0.77)
		45th SW of 2012	36.4 (0.60)	18.2 (0.43)	45.4 (0.67)
		46th SW of 2012	33.3 (0.58)	11.1 (0.33)	55.5 (0.74)
		47th SW of 2012	54.5 (0.74)	0.00 (0.01)	45.5 (0.67)
		7th SW of 2013	16.6 (0.41)	0.00 (0.01)	83.4 (0.91)
		8th SW of 2013	36.3 (0.60)	25.0 (0.50)	54.6 (0.74)
		9th SW of 2013	25.0 (0.50)	25.0 (0.50)	50.0 (0.71)
		10th SW of 2013	14.3 (0.38)	21.4 (0.46)	64.3 (0.80)
		Mean	**27.2 (0.52)[b]**	**16.7 (0.40)[b]**	**56.1 (0.74)[a]**
Chickpea	Thrissur	49th SW of 2012	60.0 (0.89)	0.00 (0.01)	40.0 (0.68)
		50th SW of 2012	47.1 (0.76)	0.00 (0.01)	52.9 (0.81)
		51st SW of 2012	72.7 (1.02)	0.00 (0.01)	27.3 (0.55)
		52nd SW of 2012	72.5 (1.02)	0.00 (0.01)	27.5 (0.55)
		1st SW of 2013	91.4 (1.27)	0.00 (0.01)	8.6 (0.28)
		Mean	**67.5 (0.82)[a]**	**0.00 (0.01)[b]**	**32.5 (0.57)[c]**

Mean of 50 observations, Values in the parenthesis are arc sin transformed values, SW- Standard week

Figures followed by same letter in the column do not differ significantly by DMRT (P=0.05)

Table 19c. Relative frequency (%) of larvae with pigmentation on lateral bands in *Helicoverpa armigera* on amaranthus and tomato in 2013 and on okra in 2014

Crop	Location	Collection period	Pigmentation on lateral bands		
			Continuous	**Continuous**	**Continuous**
Amaranthus	Thrissur	19th SW of 2013	85.8 (1.18)	0.00 (0.01)	14.2 (0.38)
		20th SW of 2013	91.7 (1.28)	0.00 (0.01)	8.30 (0.29)
		21st SW of 2013	87.5 (1.21)	0.00 (0.01)	12.5 (0.36)
		22nd SW of 2013	66.7 (0.96)	0.00 (0.01)	33.3 (0.62)
		Mean	**82.9 (0.91)[c]**	**0.01 (0.00)[b]**	**17.1 (0.41)[a]**
Tomato	Palakkad	35th SW of 2013	57.1 (0.86)	23.8 (0.51)	19.1 (0.45)
		37th SW of 2013	48.9 (0.77)	24.4 (0.52)	26.7 (0.54)
		39th SW of 2012	48.7 (0.77)	23.1 (0.50)	28.2 (0.56)
		41st SW of 2013	53.8 (0.82)	26.9 (0.54)	7.70 (0.28)
		Mean	**53.7 (0.82)[a]**	**25.3 (0.52)[b]**	**21.0 (0.47)[b]**
Okra	Thrissur	5th SW of 2014	28.6 (0.56)	23.8 (0.51)	47.6 (0.76)
		7th SW of 2014	34.8 (0.63)	8.70 (0.30)	56.5 (0.85)
		Mean	**31.7 (0.59)[b]**	**16.2 (0.41)[b]**	**52.1 (0.80)[a]**

Mean of 50 observations, Values in the parenthesis are arc sin transformed values, SW- Standard week
Figures followed by same letter in the column do not differ significantly by DMRT (P=0.05)

Helicoverpa armigera larvae collected from tomato at Thrissur had a significant variation in pigmentation on lateral bands. Continuous pigmentation on lateral bands was observed in 66.3 per cent of collected larvae, 21.6 per cent of larvae were with discontinuous pigmentation on lateral bands and no pigmentation in 12.1 per cent of larvae (Table 19a).

Helicoverpa armigera larvae collected from okra during 2012-13 showed significant variation in pigmentation on lateral bands. No pigmentation was observed on 56.1 per cent of larvae collected from okra and 27.2 per cent of larvae possessed continuous pigmentation on lateral bands and 16.7 per cent of larvae with discontinuous pigmentation on lateral bands (Table 19b). Similarly in 2014, 52.1 per cent of larve collected from okra had no pigmentation on lateral bands, whereas 31.7 per cent of larvae with continuous pigmentation on lateral bands and 16.2 per cent of larvae had discontinuous pigmentation (Table 19c).

In chickpea, 67.5 per cent of larvae possessed continuous pigmentation on lateral bands and 32.5 per cent of larvae without pigmentation on lateral bands, and no larvae with discontinuous pigmentation (Table 19b). Similarly in amaranthus 82.9 per cent of larvae with continuous pigmentation on lateral bands whereas, 17.1 per cent of larvae without pigmentation on lateral bands and no larvae with discontinuous pigmentation (Table 19c).

4.1.6. Intensity of black pigmentation on thorax and last abdominal segments of *Helicoverpa armigera* larva

Indices were worked out based on a scale (0-4) to understand the variation in black pigmentation on thorax and last abdominal segments among larval morphotypes of *H. armigera* occurring on tomato, okra, chickpea and amaranthus. In *H. armigera* morphotypes collected from tomato, the green morphotype with black lines and spots had the deepest pigmentation on thorax (1.82) and abdomen (2.52), followed by brown with white lateral lines with intensity of pigmentation on thorax,

1.13 and abdomen, 1.57, whereas other morphotypes observed on tomato had no black pigmentation on thorax and abdomen (Table 20).

Brown morphotype with dark brown lines collected from okra had pigmentation on thorax (1.42) and abdomen (1.34). In chickpea, brown morphotype with brown longitudinal lines recorded the deepest pigmentation on thorax (1.34) and abdomen (1.55), followed by green with brown longitudinal lines with intensity of pigmentation on thorax, 1.25 and abdomen, 1.42.

Among the larval morphotypes of *H. armigera* collected from amaranthus, brown with dark dorsal lines possessed the deepest pigmentation on thorax (1.85) and abdomen (2.43) followed by brown morphotype with orange spots (intensity of pigmentation on thorax, 1.12 and abdomen, 1.24), green with dark longitudinal lines (intensity of pigmentation on thorax, 0.89 and abdomen, 0.77) and light green morphotypes (intensity of pigmentation on thorax, 0.72 and abdomen, 0.88).

4.1.7. Clustering of larval morphotypes of *Helicoverpa armigera* based on multivariate analysis technique

Twenty two larval morphotypes of *H. armigera* collected from crops *viz.*, tomato, okra, chickpea and amaranthus were grouped based on morphometric parameters of larva, pupa and adult using multivariate analysis technique with IBM SPSS Version 20 statistical package. The cluster formed subsequently manifested in a dendrogram to determine grouping of morphotypes based on similarity degree of morphometric parameters.

Dendrogram analysis (Fig. 2) revealed the existence of three major morpho clusters (at Euclidian distance, 8). First cluster consisted of three morphotypes on tomato (T4-green morphotype with dark green dorsal lines, T5- green morphotype with black lines and spots T6-brown morphotype with orange spots), two morphotypes on okra (O4-greenish, O5-brownish), four morphotypes on chickpea

Table 20. Intensity of pigmentation on thoracic and last abdominal segment of *Helicoverpa armigera* larval morphotypes collected from different crops

Crop	Morphotype	Intensity of pigmentation	
		Thorax	Abdomen
Tomato	Light green	0.00 (0.71)[c]	0.00 (0.71)[c]
	Light green with orange spots	0.00 (0.71)[c]	0.00 (0.71)[c]
	Greenish	0.00 (0.71)[c]	0.00 (0.71)[c]
	Green with dark green dorsal lines	0.00 (0.71)[c]	0.00 (0.71)[c]
	Green with black lines and spots	1.82 (1.52)[a]	2.52 (1.73)[a]
	Brown with orange spots	0.00 (0.71)[c]	0.00 (0.71)[c]
	Brown with white lateral lines	1.13 (1.27)[b]	1.57 (1.43)[b]
Okra	Light green	0.00 (0.71)[c]	0.00 (0.71)[c]
	Light green with orange spots	0.00 (0.71)[c]	0.00 (0.71)[c]
	Yellowish green	0.00 (0.71)[c]	0.00 (0.71)[c]
	Green	0.00 (0.71)[c]	0.00 (0.71)[c]
	Brown	0.00 (0.71)[c]	0.00 (0.71)[c]
	Brown with dark brown lines	1.39 (1.42)[a]	1.36 (1.34)[a]
Chickpea	Green with green longitudinal lines	0.00 (0.71)[c]	0.00 (0.71)[c]
	Greenish	0.00 (0.71)[c]	0.00 (0.71)[c]
	Green with brown longitudinal lines	1.25 (1.32)[a]	1.42 (1.37)[a]
	Brown with brown longitudinal lines	1.36 (1.34)[a]	1.55 (1.43)[a]
Amaranthus	Light green	0.00 (0.71)[c]	0.00 (0.71)[c]
	Greenish	0.72 (1.10)[b]	0.88 (1.74)[b]
	Green with dark longitudinal lines	0.89 (1.17)[b]	0.77 (1.12)[b]
	Brown with orange spot	1.12 (1.27)[b]	1.24 (1.30)[b]
	Brown with dark dorsal lines	1.85 (1.53)[a]	2.43 (1.71)[a]

Mean of 50 observations, Values in the parenthesis are square root transformed values

Figures followed by same letter in the raw do not differ significantly by DMRT (P=0.05)

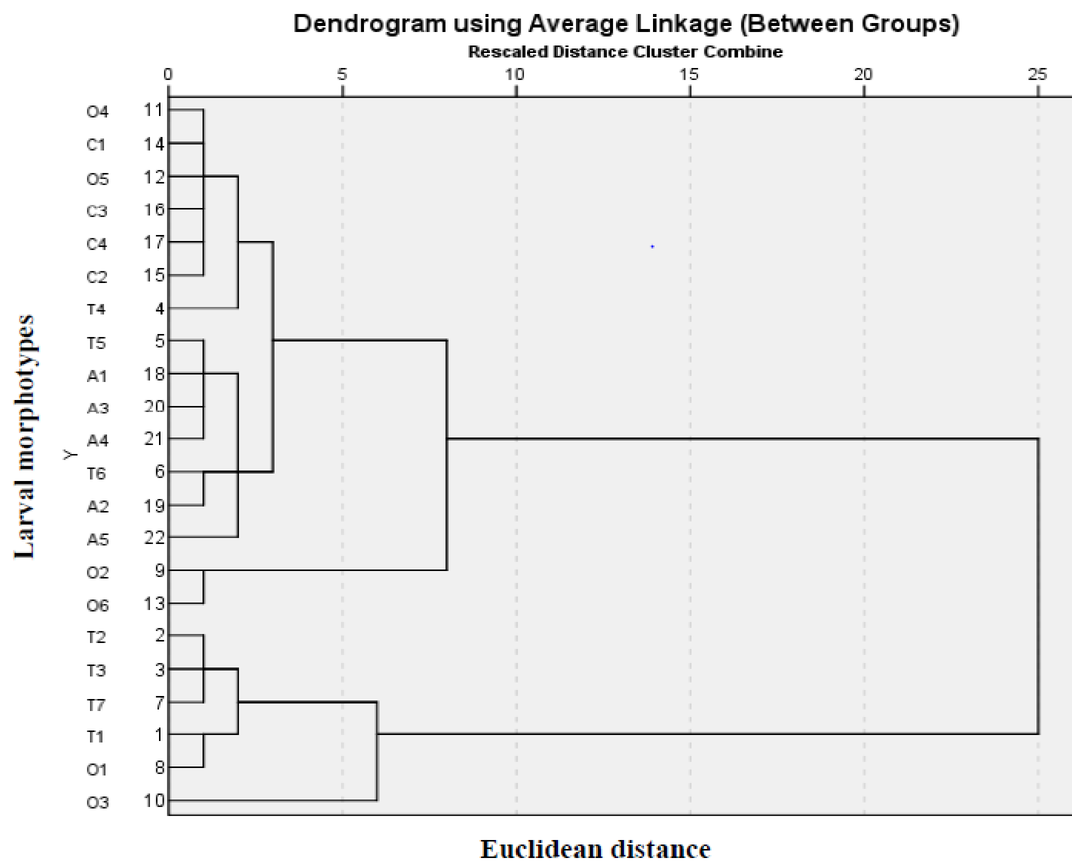


Fig 2. Dendrogram of *Helicoverpa armigera* larval morphotypes occurring on different crops based on multivariate analysis technique

(T1-T7: Larval morphotypes on tomato, O1-O6: Larval morphotypes on okra, C1-C4: Larval morphotypes on chickpea, A1-A5: Larval morphotypes on amaranthus)

(C1-green with green longitudinal lines, C2-greenish, C3-green with brown longitudinal lines, C4-brown with brown longitudinal lines and five morphotypes on amaranthus (A1-green with dark longitudinal lines, A2-greenish, A3-green with dark longitudinal lines, A4- brown with orange spots, A5-brown with dark dorsal lines).

Second cluster consisted of two morphotypes on okra (O2- light green with orange spots and O6- brown with dark brown longitudinal lines). Four morphotypes on tomato (T1-light green, T2- light green with orange spots, T3-greenish and T7- brown with white lateral lines) and two morphotypes on okra (O1-light green and O3- yellowish green) were grouped together in third cluster.

At Euclidean distance 25, twenty larval morphotypes were grouped in to two major clusters. First cluster contained sixteen morphotypes belonged to first two clusters, whereas second cluster consisted of six larval morphotypes belonged to third cluster.

4.2. Molecular characterization of *Helicoverpa armigera* larval morphotypes

4.2.1. Isolation of larval genomic DNA

Genomic DNA from twenty two larval morphotypes of *H. armigera* collected from crops *viz*., tomato, okra, chickpea and amaranthus were isolated through modified CTAB method and intact bands were obtained when resolved at 0.8 per cent agarose gel (Plate 9). Spectrophotometric analysis gave ratio of UV absorbance (A260/A280) between 1.80 and 1.94 (Table 21) indicating the good quality genomic DNA.

Table 21. Quality and quantity of DNA extracted from *Helicoverpa armigera* larval morphotypes determined by Nanodrop method

Crop	*H. armigera* Larval morphotypes	A260nm	A280nm	A260/280	Quantity (ng/µl)
Tomato	Light green	5.454	2.977	1.83	1272.6
	Light green with orange spots	10.26	5.362	1.91	512.98
	Greenish	0.471	0.254	1.85	823.53
	Green with dark green dorsal lines	1.185	0.650	1.82	590.25
	Green with black lines and spots	3.400	1.885	1.80	970.02
	Brown with orange spots	0.245	0.219	1.90	512.26
	Brown with white lateral lines	76.28	41.07	1.86	3814.3
Okra	Light green	34.24	18.30	1.87	1712.0
	Light green with orange spots	6.984	3.578	1.86	349.18
	Yellowish green	3.574	1.90	1.88	647.09
	Green	2.509	1.376	1.82	1512.4
	Brown	1.731	0.928	1.87	886.56
	Brown with dark brown lines	1.545	0.805	1.92	869.84
Chickpea	Green with green longitudinal lines	0.595	0.306	1.94	829.73
	Greenish	4.531	2.463	1.84	901.79
	Green with brown longitudinal lines	3.487	1.885	1.85	887.03
	Brown with brown longitudinal lines	3.720	2.00	1.86	756.93
Amaranthus	Light green	0.973	0.521	1.87	348.64
	Greenish	1.043	0.570	1.83	752.14
	Green with dark longitudinal lines	1.158	0.611	1.90	357.90
	Brown with orange spot	1.268	0.611	1.92	863.42
	Brown with dark dorsal lines	1.396	0.705	1.83	769.82

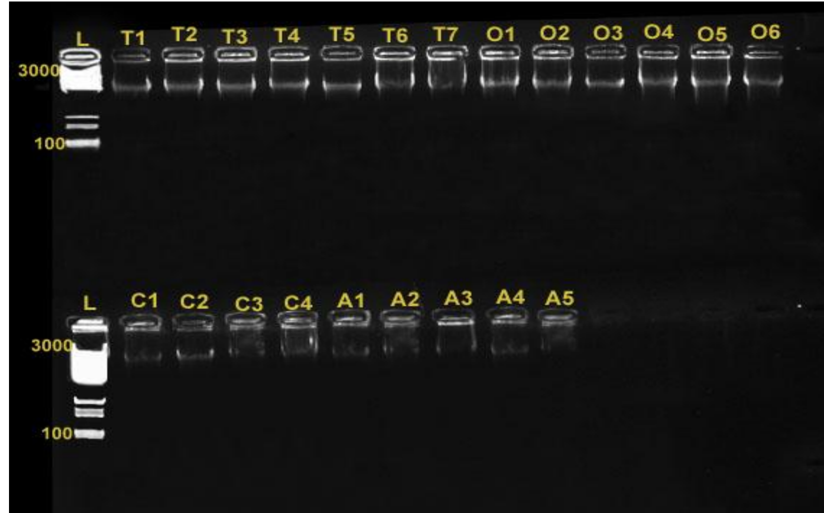


Plate 9. Agarose gel electrophoresis of genomic DNA of *Helicoverpa armigera* larval morphotypes occurring on tomato, okra, chickpea and amaranthus (L: Ladder [3 kb], lane T1-T7: larval morphotypes on tomato, lane O1-O5: larval morphotypes on okra, lane C1-C4: larval morphotypes on chickpea, lane A1-A5: larval morphotypes on amaranthus)

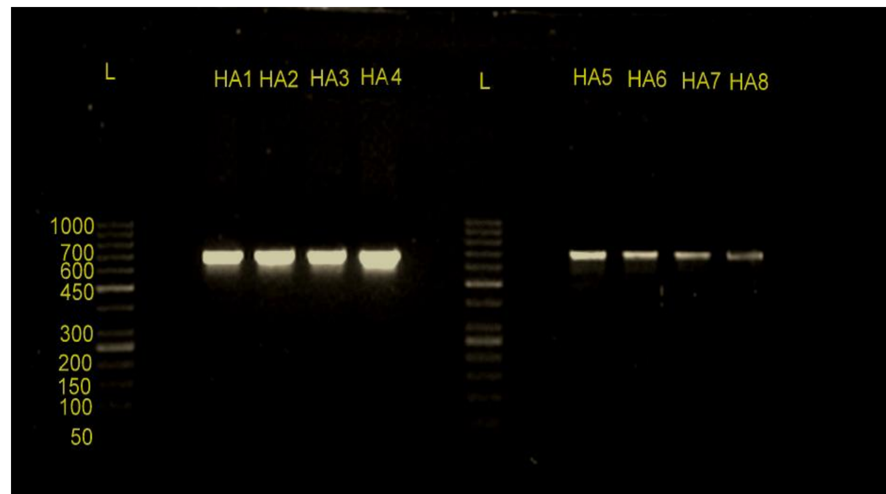


Plate 10. Agarose gel electrophoresis of genomic DNA isolated from *Helicoverpa armigera* upon amplification with mtCO1 primer. (L: 50 bp ladder, H1-H8: *Helicoverpa* DNA samples)

4.2.2. DNA barcoding of *Helicoverpa armigera*

The mtCO1 region was amplified with universal DNA bacode primer in a thermal cycler and the PCR product gave an intact band at 700 bp when resolved at 1 per cent agarose gel (Plate 10). The mtCO1sequence generated from tomato fruit borer consisted of 608 bp and homology of sequence with other reported sequences were analyzed. The sequence showed significant homology to *H. armigera* mitochondrial Cytochrome Oxidase CO1 gene already deposited in the public domain database using 'blast n' search tool. The blast results showed 100 per cent query coverage and 99 per cent identity to *H. armigera* mtCO1gene. Then the sequence was aligned and annotated using bioinformatics tools, BioEdit and MEGA6. The sequences thus obtained were submitted to BankIt, NCBI under the accession number KM403206 and KP210095.

An account was opened in workbench session of BOLD systems v3 database and a new project 'RMTKL'was created. Specimen data *viz*., specimen identifiers, specimen taxonomy, specimen details, collection details was submitted and an auto generated process ID 'RMTKL001-14' was obtained. Further, primer details, high resolution specimen images, mitochondrial DNA sequences (fasta) and the trace files (.ab1) obtained from sequencer were uploaded to the database and the corresponding barcode of *H. armigera* (Plate 11) was generated. Upon verification of DNA sequences submitted, the database allotted barcode index number (BIN), BOLD: AAA5223. Altogether 560 sequences of *H. armigera* were coming under the allotted BIN. Based on the distance model kimura 2 parameter, a BOLD taxon ID tree (Fig. 3) was constructed in database. It showed that the nearest neighborhood of our sequence was *H. armigera* (Unpublished ID) sequence deposited from India. However, the sequences of *H. armigera* deposited in database from Punjab, Maharashtra, South Africa, United Kingdom, Kenya, Pakistan, Brazil, Italy and Germany also shared similarity with our sequences.

Coord. Source:

Coord. Accuracy:

Depth:

Depth Accuracy :

SEQUENCE: COI-5P [Funding Source: N/A]

Sequence ID: RMTKL001-14.COI-5P

GenBank Accession: KP210095

Last Updated: 2015-03-22

Genome: Mitochondrial

Locus: Cytochrome Oxidase Subunit 1 5' Region

Nucleotides: 608 bp

```
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GAGCAGAATTAGGTAATCCTGGATCTTTAATTGGAGATGATCAAATTTATAATACTATTGTTACAGCTCATGCTT
TTATTATAATTTTTTTTATAGTTATACCAATTATAATTGGTGGATTTGGTAATTGACTTGTACCTTTAATATTAG
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ATGGAGGAAGATCAGTAGATCTAGCTATTTTTTCTTTACATTTAGCTGGAATCTCATCTATTTTAGGAGCAATTA
ATTTTATTACTACTATTATTAATATAAAATTAAATAGCTTATCTTTTGATCAAATACCATTATTTATTTGAGTTG
TAGGAATTACTGCATTTTTATTATTATTATCATTACCAGTTTTAGCAGGTGCTATTACTATACTTTTAACAGATC
GAAACCTT
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Amino Acids:

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APDMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGTGWTVYPPLSSNIAHGGSSVDLAIFSLHLAGISSILGAIN
FITTIINMKLNSLSFDQMPLFIWVVGITAFLLLLSLPVLAGAITMLLTDRNL
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Illustrative Barcode:

0 268

269 537

538 607

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Add Tags & Comments Comments: 0 Associated Tags: No Tags

Plate 11. DNA barcode of *Helicoverpa armigera* generated by BOLD systems v3

Fig 3. A section of BOLD ID tree constructed in database

4.2.3. Molecular marker analysis

Thirteen primer sets were screened for amplification of SSR region in genomic DNA of *H. armigera* larval larval morphotypes with thermal cycler correspondingly programmed primer specific annealing temperature. The amplification patterns obtained were analyzed (Plate 12, 13 and 14) and eight primer sets were selected for SSR characterization of *H. armigera* (Table 22).

DNA extracted from twenty two *H. armigera* larval morphotypes were amplified with eight selected SSR primers, the amplified products were resolved in 10 per cent poly acrylamide gel electrophoresis, visualized using silver stain method and photographed. Details on amplification of SSR region in genomic DNA of *H. armigera* larval morphotypes occurring on different crops are given below.

***Helicoverpa armigera* larval morphotypes on tomato**

The primer HaSSR1 produced two monomorphic bands (at 150 bp and 550bp) in all larval morphotypes on tomato, whereas a distinct faint band at 850 bp was produced in both light green larvae and light green with orange spots and at 800 bp in both light green and brown larval morphotypes with white lateral lines (Plate 15a).

HaSSR2 yielded two unique bands (at 400 bp and 450 bp) in both greenish and green with black lines and spots larva (at 150 bp and 210 bp) (Plate 15b). Whereas three distinct bands were produced in light green (at 250 bp, 800 bp and 900 bp) and light green with orange spots (at 100bp, 700bp and 800bp), two bands in green with dark green dorsal lines and brown with orange spots (at 100bp and 700 bp), two bands in greenish (at 100 bp and 250 bp.), green with black lines and spots (250 bp and 700 bp) and four bands in brown with white lateral lines (at 100 bp, 250 bp, 800 bp and 900 bp).

The primer HaSSR3 gave a unique band (at 800 bp) in greenish morphotype and three unique bands (at 130 bp, 140 bp and 175 bp) in brown with white lateral

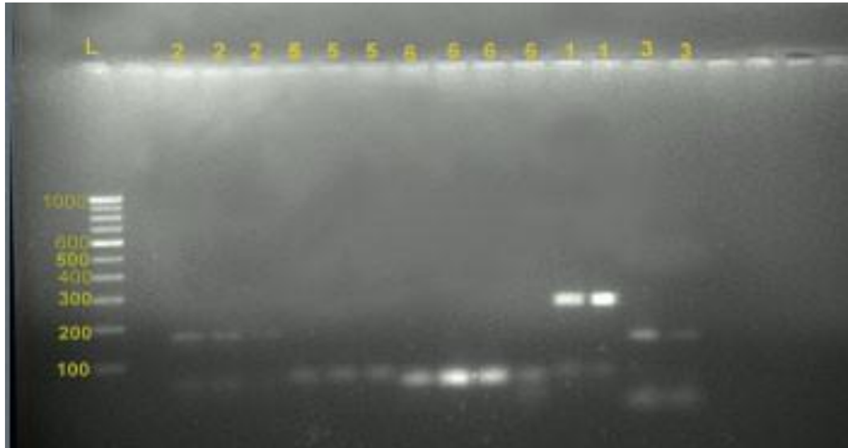


Plate 12. Screening of SSR primers (HaSSR1, HaSSR2, HaSSR3, HaSSR5 and HasSSR6) [L: 100 bp ladder, 1=HaSSR1, 2=HaSSR2, 3=HaSSR3, 5=HaSSR5 and 6=HaSSR6]

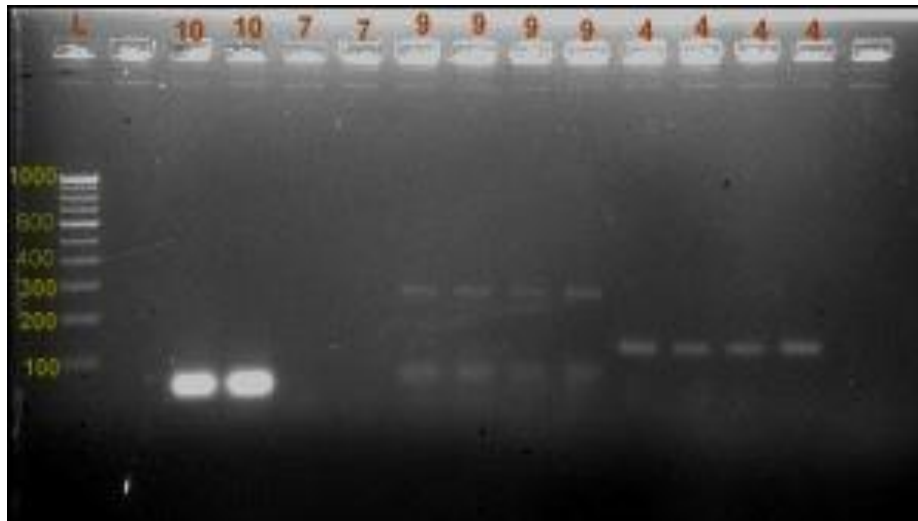
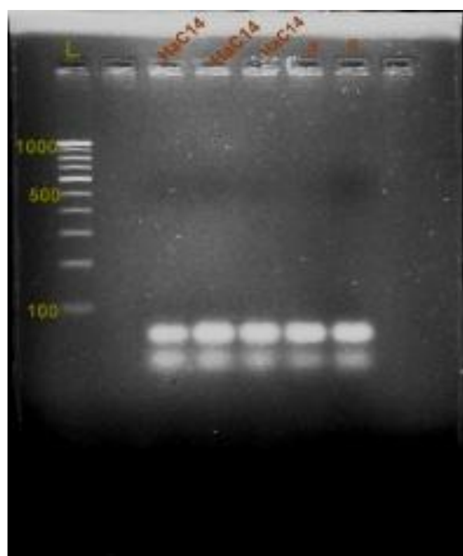


Plate 13. Screening of SSR primers (HaSSR4, HaSSR7, HaSSR9 and HaSSR10) [L: 100 bp ladder, 4=HaSSR4, 7=HaSSR7, 9=HaSSR9, 10=HaSSR10]



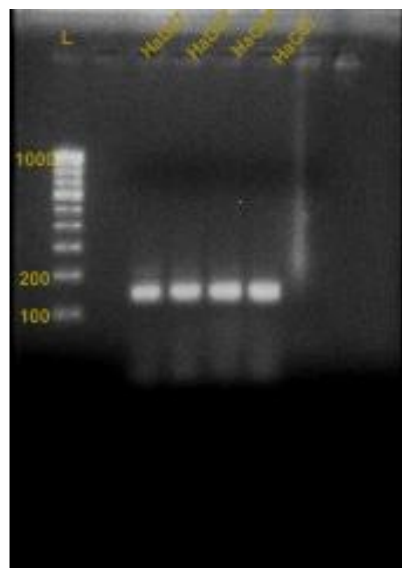


Plate 14. Screening of SSR primers (HaSSR8, HaD47, HaC14 and HaC87)

[L: 100 bp ladder, 8=HaSSR8, HaD47, HaC14 and HaC87]

Table 22. Amplification pattern of SSR primers in *Helicoverpa armigera* larval morphotypes

Sl No	Primer	No of bands	Amplification pattern		Remarks
			Distinct	Faint	
1	HaSSR1	1	1	0	Selected
2	HaSSR2	1	1	0	Selected
3	HaSSR3	1	1	0	Selected
4	HaSSR4	1	1	0	Selected
5	HaSSR5	0	0	0	--
6	HaSSR6	0	0	0	--
7	HaSSR7	1	1	0	Selected
8	HaSSR8	0	0	0	--
9	HaSSR9	1	1	0	Selected
10	HaSSR10	0	0	0	--
11	HaD47	1	1	0	Selected
12	HaC14	1	0	0	--
13	HaC87	1	1	0	Selected

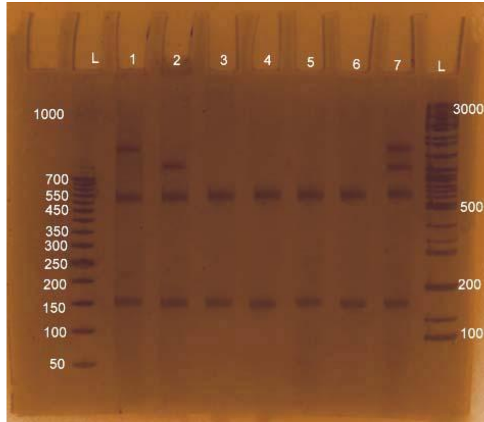
lines. The primer produced two monomorphic bands (at 230 bp and 220bp) in all larval morphotypes. In addition, it produced a single distinct band (at 100 bp) in light green, light green with orange spots and brown with orange spots, two bands (at 400 bp and 350 bp) in green with black lines and spots, three bands (at 400 bp 350 bp and 100 bp) in greenish, green with dark green dorsal lines and brown with white lateral lines (Plate 15c).

However, HaSSR4 yielded two unique bands (at 150bp and 200 bp) in light green morphotype. It also produced three monomorphic bands (at 100 bp, 300 bp and 800 bp) in all larval morphotypes. In addition, it produced distinct single band (at 175 bp) in brown with orange spots, two bands (at 175 bp and 325 bp) in light green with orange spots and greenish larval morphotypes and light green larval morphotypes (at 130 bp, and 325 bp) (Plate 15d).

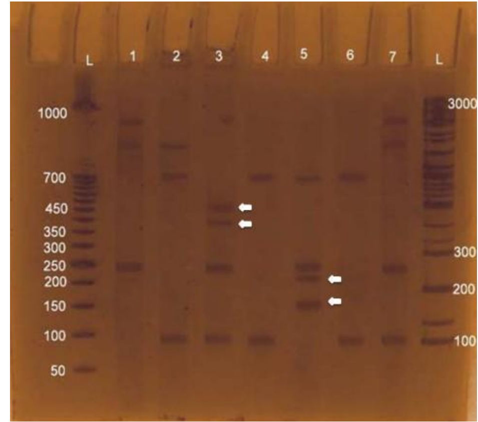
Single monomorphic band at 50 bp in all larval morphotypes was produced by HaSSR7. In addition, it produced single distinct band in light green with orange spots (at 150 bp) and T3 (at 100 bp) whereas, two bands in light green (at 100 bp and 15 bp) (Palte. 15e) . HaSSR9 and HaD47 produced monomorphic band at 50 bp in all larval larval morphotypes collected from tomato (Plate 15f; Plate 15g). Similarly HaC87 yielded single band for all larval larval morphotypes at 100 bp (Plate 15h).

Fingerprint of *H. armigera* larval morphotypes occurring on tomato developed based on presence of clear and distinct bands and molecular weight of bands produced with eight selected SSR primers (Fig. 4). Ten bands shared among all larval morphotypes were indicated by green colour code, whereas the bands shared among six, five, four, three and two larval morphotypes were indicated by blue, yellow, orange, grey and violet color code respectively. However, the unique bands present in larval morphotypes were indicated in red colour code.

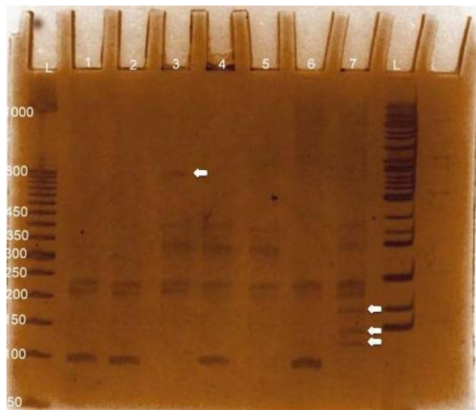
Among the larval morphotypes occurring on tomato, two unique bands present in both light green (HaSSR4) and green colour morph with black lines and



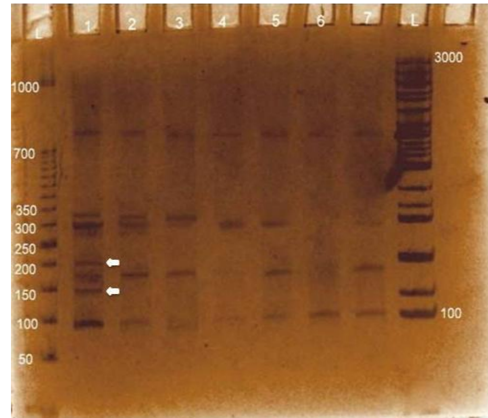
a. HaSSR1



b. HaSSR2

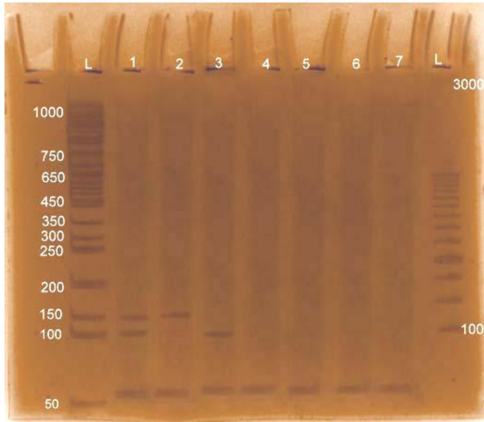


c. HaSSR3

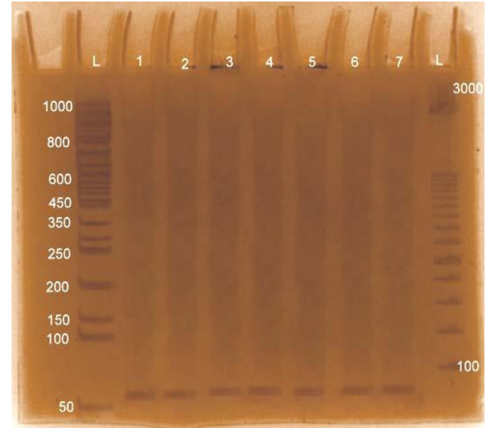


d. HaSSR4

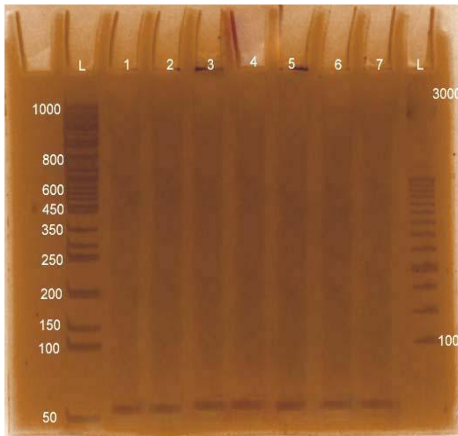
Plate 15. Simple sequence repeat fragments generated from seven *Helicoverpa armigera* larval morphotypes (lane 1to lane 7) occuring on tomato upon amplification with HaSSR1, HaSSR2, HaSSR3 and HaSSR4 when resolved in PAGE (10%)



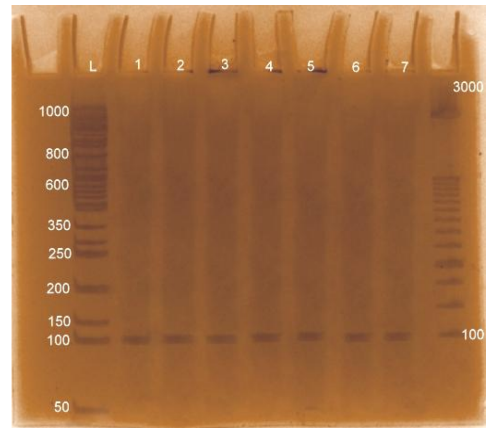
e. HaSSR7



f. HaSSR9



g. HaD47



h. HaC87

Plate 15. Simple sequence repeat fragments generated from seven *Helicoverpa armigera* larval morphotypes occuring on tomato (lane 1to lane 7) upon amplification with HaSSR7, HaSSR9, HaD47 and HaC87 when resolved in PAGE (10%)

Fig. 4. DNA fingerprint of *Helicoverpa armigera* larval morphotypes occurring on tomato

Primer	1	2	3	4	5	6	7	8
Mol. Size (bp)								
1000								
900								
850								
800								
550								
325								
300								
250								
230								
220								
200								
175								
150								
100								
50								

1. Light green

Primer	1	2	3	4	5	6	7	8
Mol. Size (bp)								
1000								
900								
800								
700								
550								
325								
300								
230								
220								
200								
175								
150								
100								
50								

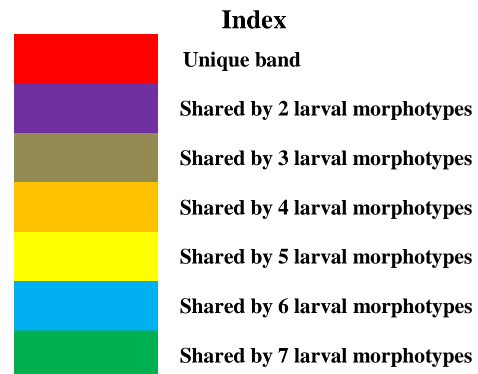
2. Light green with orange spots

Primer	1	2	3	4	5	6	7	8
Mol. Size (bp)								
1000								
800								
550								
450								
400								
350								
325								
300								
250								
230								
220								
200								
175								
150								
100								
50								

3. Green

Primer	1	2	3	4	5	6	7	8
Mol. Size (bp)								
1000								
900						-		
800								
700								
550								
400								
350								
300								
250								
230								
220								
175								
150								
100								
50								

4. Green with dark green dorsal lines



Primers: 1- HaSSR1, 2- HaSSR2, 3-HaSSR3, 4-HaSSR4, 5- HaSSR7, 6-HaSSR9, 7-HaD47, 8-HaC87

Fig. 4. DNA fingerprint of *Helicoverpa armigera* larval morphotypes occurring on tomato

Primer	1	2	3	4	5	6	7	8
Mol. Size (bp)								
1000								
800								
700								
550								
400								
350								
325								
300								
250								
230								
220								
210								
175								
150								
100								
50								

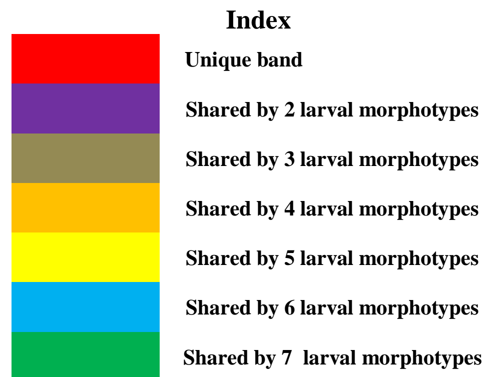
5. Green with black lines and spots

Primer	1	2	3	4	5	6	7	8
Mol. Size (bp)								
1000								
900								
800								
700								
550								
400								
350								
300								
250								
230								
220								
175								
150								
100								
50								

6. Brown with orange spots

Primer	1	2	3	4	5	6	7	8
Mol. Size (bp)								
1000								
900								
850								
800								
550								
400								
350								
300								
250								
230								
220								
175								
150								
140								
130								
100								
50								

7. Brown with white lateral lines



Primers: 1- HaSSR1, 2- HaSSR2, 3-HaSSR3, 4-HaSSR4, 5- HaSSR7, 6-HaSSR9, 7-HaD47, 8-HaC87

spots (HaSSR2), three bands in greenish larval morphotypes (HaSSR2, HaSSR3) and four bands in brown colour morph with white lateral lines (HaSSR1, HaSSR3).

***Helicoverpa armigera* larval morphotypes on okra**

The primer HaSSR1 produced two unique bands (at 350 bp and 500 bp) in brown with dark brown longitudinal lines morphotype. It also produced a monomorphic band at 120 bp in all larval morphotypes, whereas a single faint distinct band at 300 bp in yellowish green and brownish and brown with dark brown dorsal lines morphotypes (Plate 16a). HaSSR2 gave a unique band in both yellowish green (at 220 bp) and greenish (850 bp) morphotypes. It produced a single band at 100 bp in light green, light green with orange spots and brown with dark brown longitudinal lines. In addition to the unique band, HaSSR2 produced two bands (at 200 bp and 275 bp) in yellowish green and three bands (at 100bp, 275 bp and 700 bp) in greenish morphotype. The primer gave four bands (at 100 bp, 200 bp, 275 bp and 700 bp) in brownish morphotype (Plate 16b).

The primer HaSSR3 had not produced unique bands in *H. armigera* morphotypes on okra. However, it produced a single band at 50 bp in light green with orange spots, greenish, brownish, and brown with dark brown dorsal lines and at 150 bp in light green and yellowish green larval morphotypes (Plate 16c), whereas, HaSSR4 yielded a monomorphic band at 50 bp in all larval morphotypes and a single distinct band at 150 bp in light green with orange spots (Plate 16d).

A unique band at 100 bp in brownish morphotype was produced by HaSSR7. In addition it also produced a single band at 50 bp. In other morphotypes viz., light green, light green with orange spots, yellowish green and greenish, the primer yielded a single band at 50 bp. HaSSR7 had not produced bands in brown with dark brown longitudinal lines (Plate 16e).

The primer, HaSSR9 yielded a unique band in both light green with orange spots (800 bp) and brown with dark brown dorsal lines (250 bp) and monomorphic band at 50 bp in all larval morphotypes (Plate 16f).

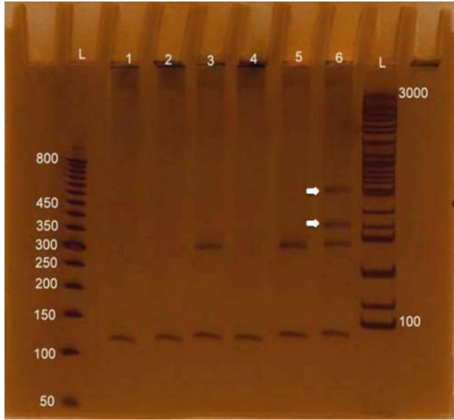
HaD47 produced a unique band in both brownish (at 150 bp) and brown with dark brown longitudinal lines (at 250 bp) and three unique bands (at 150 bp, 400 bp and 800 bp) in light green with orange spots. It also produced a monomorphic band at 50 bp in all larval morphotypes (Plate 16g). The primer HaC87 produced a unique band in light green morphotype along with band at 50 bp, whereas a single distinct band in yellowish green (at 175 bp) and brown with dark brown lines (50 bp) and two bands (at 50 bp and 175 bp) in greenish and brownish morphotypes (Plate 16h).

Finger print of *H. armigera* larval morphotype occurring on okra based on eight SSR primers was presented in fig. 5. Four bands produced by HaSSR1, HaSSR4, HaSSR9, HaD47 and HaC87 in all larval morphotypes were represented in green colour code. Similarly, the bands shared among five, four, three and two larval morphotypes were indicated by blue, orange, grey and violet colour code, and however unique bands present in larval morphotypes were indicated by red colour code.

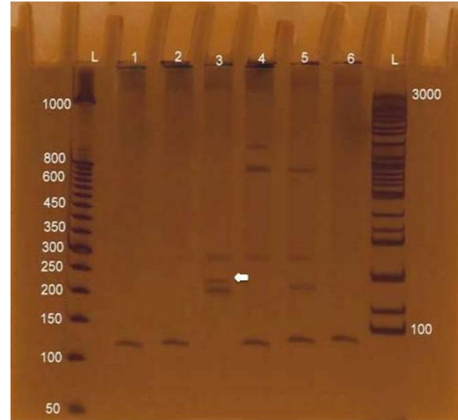
It was observed that a unique band present in light green (HaC87) , yellowish green (HaSSR2) and greenish larval morphotypes (HaSSR2), two bands in brownish larval morphotypes (HaSSR7, HaD47), three bands in brown with dark brown dorsal lines (HaSSR1, HaSSR9) and four bands in light green with orange spots (HaSSR9, HaD47).

Helicoverpa armigera **larval morphotypes on chickpea**

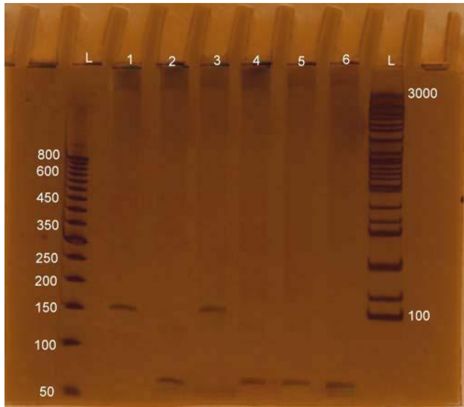
The primer HaSSR1 produced a unique band at 800 bp in green with brown longitudinal lines. It produced two monomorphic bands (at 100 bp and 300 bp) in all larval morphotypes. In addition, it gave single band (at 600 bp) in green with brown



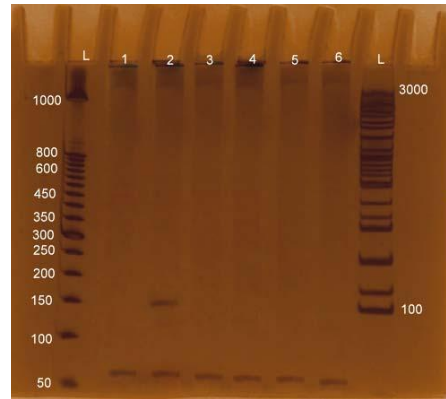
a. HaSSR1



b. HaSSR2

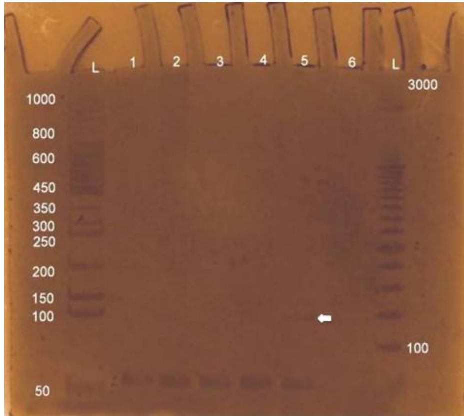


c. HaSSR3

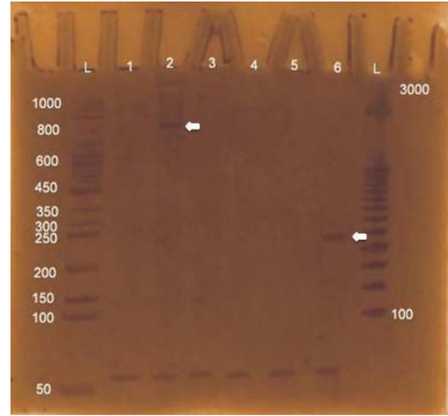


d. HaSSR4

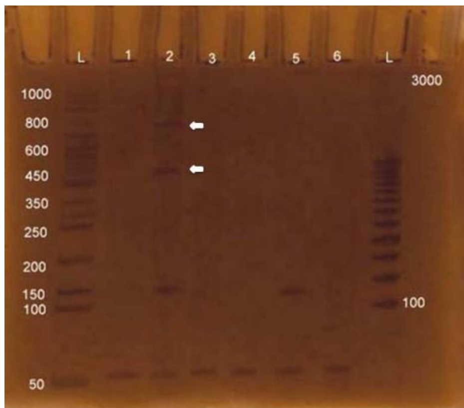
Plate 16. Simple sequence repeat fragments generated from six *Helicoverpa armigera* larval morphotypes occuring on okra (lane1 to lane6) upon amplification with HaSSR1, HaSSR2, HaSSR3 and HaSSR4 when resolved in PAGE (10%)



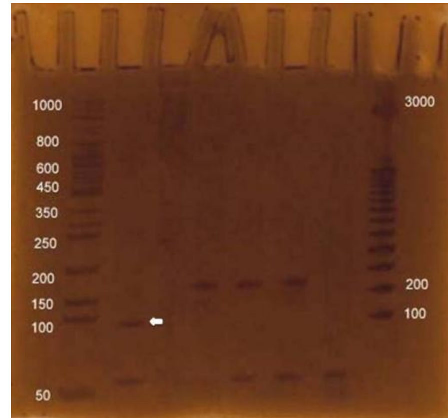
e. HaSSR7



f.HaSSR9



g. HaD47



h. HaC87

Plate 16. Simple sequence repeat fragments generated from six *Helicoverpa armigera* larval morphotypes occuring on okra (lane 1to lane 6) upon amplification with HaSSR7, HaSSR9, HaD47 and HaC87 when resolved in PAGE (10%)

Fig. 5. DNA fingerprint of *Helicoverpa armigera* larval morphotypes occurring on okra

Primer	1	2	3	4	5	6	7	8
Mol. Size (bp)								
150								
120								
100								
50								

1. Light green

Primer	1	2	3	4	5	6	7	8
Mol. Size (bp)								
800								
450								
150								
120								
100								
50								

2. Light green with orange spots

Primer	1	2	3	4	5	6	7	8
Mol. Size (bp)								
300								
275								
220								
200								
175								
150								
120								
50								

3. Yellowish green

Primer	1	2	3	4	5	6	7	8
Mol. Size (bp)								
850								
700								
275								
175								
120								
100								
50								

4. Green

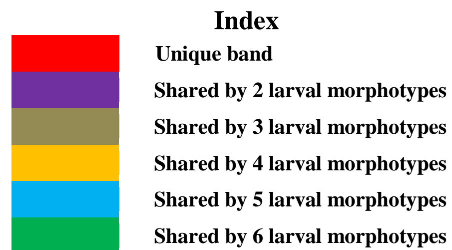
Primer	1	2	3	4	5	6	7	8
Mol. Size (bp)								
700								
300								
275								
200								
175								
150								
120								
100								
50								

5. Brown

Primer	1	2	3	4	5	6	7	8
Mol. Size (bp)								
500								
350								
300								
250								
120								
100								
50								

6. Brown with dark brown longitudinal lines

Index



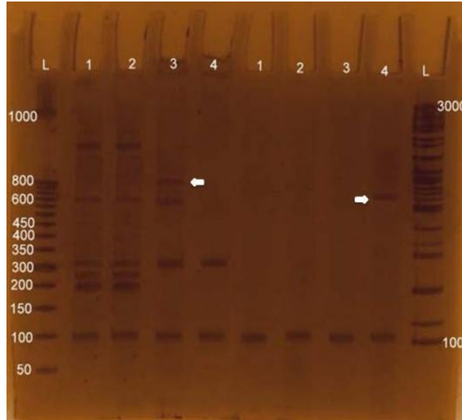
Primers: 1- HaSSR1, 2- HaSSR2, 3-HaSSR3, 4-HaSSR4, 5- HaSSR7, 6-HaSSR9, 7-HaD47, 8-HaC87

longitudinal lines, four bands (at 200 bp, 250 bp, 600 bp and 900 bp) in both greenish and green with green dorsal lines (Plate 17a). Whereas, HaSSR2 yielded a unique band (at 600 bp) in brown with brown longitudinal lines. However, it produced a monomorphic band at 100 bp in all larval morphotypes on chickpea (Plate 17a).

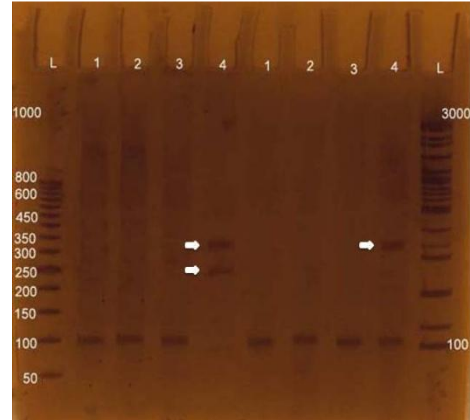
HaSSR3 gave two unique bands (at 250 bp and 325 bp) in brown with brown longitudinal lines, whereas, it produced band at 100 bp in all morphotypes on chickpea except brown with brown longitudinal lines (Plate 17b). The primer HaSSR4 produced a unique band at 300 bp in green with brown longitudinal lines. It also produced a monomorphic band at 100 bp in all morphotypes on chickpea (Plate 17b). HaSSR7 produced a unique band in both green with brown longitudinal lines and brown with brown longitudinal lines. Apart from it also gave monomorphic band at 100 bp in all larval morphptypes on chickpea. However, it produced three bands (at 300 bp, 600 bp and 900 bp) in green with green dorsal lines and greenish morphotypes (Plate 17c).

HaSSR9 gave two unique bands (at 850 bp and 900 bp) in brown with brown longitudinal lines along with a monomorphic band 100 bp in all larval morphotypes on chickpea (Plate 17c). HaD47 yielded a unique band at 600 bp in both green with brown longitudinal lines and brown with brown longitudinal lines. It also produced monomorphic band at 100 bp in all larval morphotypes and four bands at 200 bp. 220bp, 600 bp and 900 bp) in both green with green dorsal lines and greenish morphotypes (Plate 17d). HaC87 produced two unique bands (at 650 bp and 800 bp) in brown with brown longitudinal lines and two monomorphic bands (at 100 bp and 150 bp) in all morphotypes on chickpea (Plate 17d).

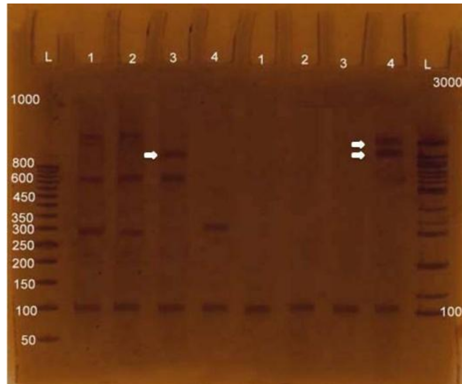
Finger print of *H. armigera* larval morphotypes occurring on chickpea was developed based on eight SSR primers (Fig. 6). Nine bands produced by HaSSR1, HaSSR2, HaSSR4, HaSSR7, HaSSR9, HaD47 and HaC87 in all larval morphotypes were represented in green colour code. The bands shared among three and two larval



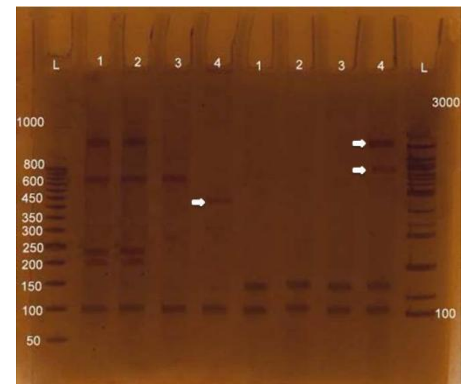
a. HaSSR1 HaSSR2



b. HaSSR3 HaSSR4



c. HaSSR7 HaSSR9



d. HaD47 HaC87

Plate 17. Simple sequence repeat fragments generated from four *Helicoverpa armigera* larval morphotypes occuring on chickpea (lane 1to lane 4) upon amplification with HaSSR1, HaSSR2, HaSSR3, HaSSR4, HaSSR7, HaSSR9, HaD47 and HaC87 when resolved in PAGE (10%)

Fig. 6. DNA fingerprint of *Helicoverpa armigera* larval morphotypes occurring on chickpea

Primer	1	2	3	4	5	6	7	8
Mol. Size (bp)								
900								
600								
300.								
250								
220								
200								
150								
100								

1. Green with green longitudinal lines

Primer	1	2	3	4	5	6	7	8
Mol. Size (bp)								
900								
600								
300								
250								
220								
200								
150								
100								

2. Green

Primer	1	2	3	4	5	6	7	8
Mol. Size (bp)								
850								
800								
600								
300								
150								
100								

3. Green with brown longitudinal lines

Primer	1	2	3	4	5	6	7	8
Mol. Size (bp)								
900								
850								
650								
600								
400								
325								
300								
250								
150								
100								

4. Brown with brown longitudinal lines

Index

Unique band

Shared by 2 larval morphotypes

Shared by 3 larval morphotypes

Shared by 4 larval morphotypes

Fig 7. DNA finger print of *Helicoverpa armigera* colour morphs occurring on amaranthus

Primer	1	2	3	4	5	6	7	8
Mol. Size (bp)								
150								
50								

1. Light green

Primer	1	2	3	4	5	6	7	8
Mol. Size (bp)								
900								
850								
250								
200								
100								
50								

2. Green

Primer	1	2	3	4	5	6	7	8
Mol. Size (bp)								
250								
150								
100								
50								

3. Green with dark dorsal lines

Primer	1	2	3	4	5	6	7	8
Mol. Size (bp)								
325								
200								

5. Br own with orange spots

Primer	1	2	3	4	5	6	7	8
Mol. Size (bp)								
250								
150								
125								
100								
50								

4. Brown with dark dorsal lines

Index

Unique band

Shared by 2 larval morphotypes

Shared by 3 larval morphotypes

Shared by 4 larval morphotypes

Shared by 5 larval morphotypes

Primers: 1- HaSSR1, 2- HaSSR2, 3-HaSSR3, 4-HaSSR4, 5- HaSSR7, 6-HaSSR9, 7-HaD47, 8-HaC87

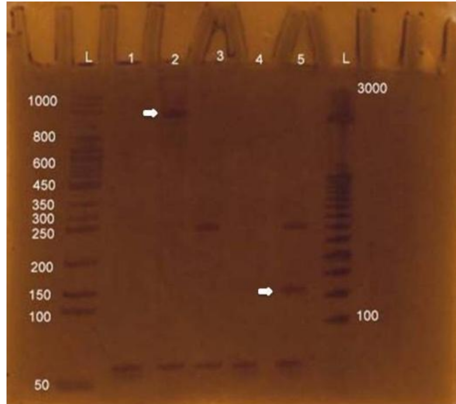
morphotypes were indicated by grey and violet colour code, and however unique bands present in larval morphotypes were indicated by red colour code.

Among the larval morphotypes occurring on chickpea, four unique bands present in green with brown dorsal lines (HaSSR1, HaSSR4, HaSSR7 and HaD47) and nine unique bands (HaSSR2, HaSSR3, HaSSR7, HaSSR9, HaD47 and HaC87) present in brown with brown dorsal lines. However green colour morph with green dorsal lines and greenish larval morphotypes did not possess unique bands.

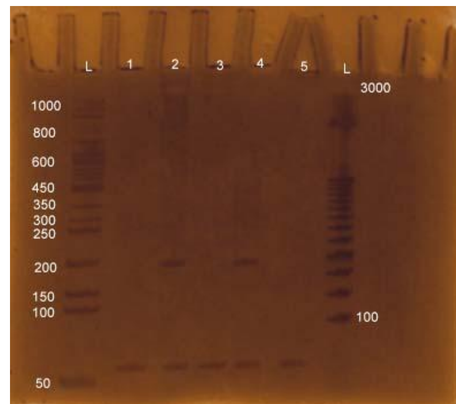
***Helicoverpa armigera* larval morphotypes on amaranthus**

HaSSR1 produced a unique band in both greenish (at 850 bp) and brown with dark dorsal lines (150 bp) larval morphotypes, whereas it gave a monomorphic band at 50 bp in morphotypes. However a single distinct band at 250 bp was produced in both green with dark dorsal lines and brown with dark dorsal lines (Plate 18a). The primer HaSSR2 yielded a unique band at 200 bp in both greenish and brown with orange spots. It also produced a monomorphic band at 50 bp in all morphotypes (Plate 18b). However, HaSSR3 did not produce any unique bands in orphotypes on amaranthus. But, it produced a monomorphic band at 50 bp in all larval morphotypes and a single distinct band at 150 bp in light green and green colour morph with dark dorsal lines (Plate 18c).

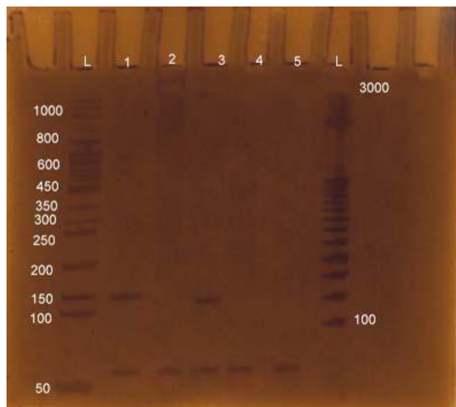
The primer HaSSR4 gave a unique band (at 100 bp) in brown with dark dorsal lines and three unique bands (at 200 bp, 250 bp and 900 bp) in greenish morphotypes. It produced a monomorphic band at 50 bp in all larval morphotypes (Plate 18d). HaSSR7 produced a uniqe band at 850 bp in greenish morphotypes and monomorphic band at 50 bp in all morphotypes on amaranthus (Plate 18e). However, HaSSR9 gave unique band at 325 bp in brown with orange spots and monomorphic band at 50 bp in all morphotypes (Plate 18f).



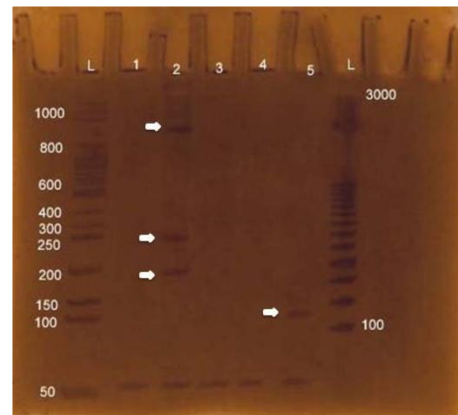
a. HaSSR1



b. HaSSR2

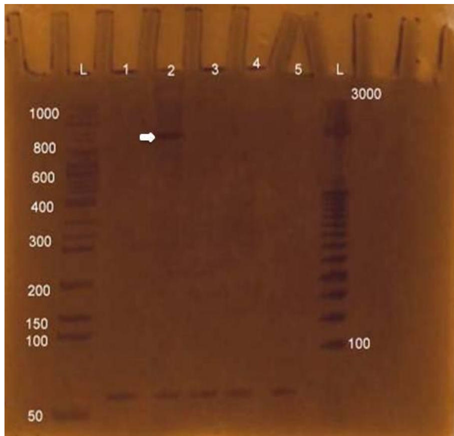


c.HaSSR3

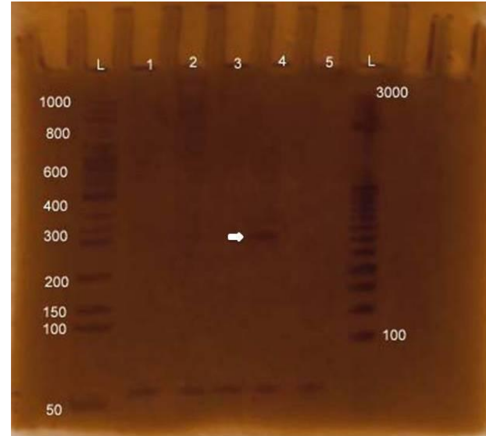


d. HaSSR4

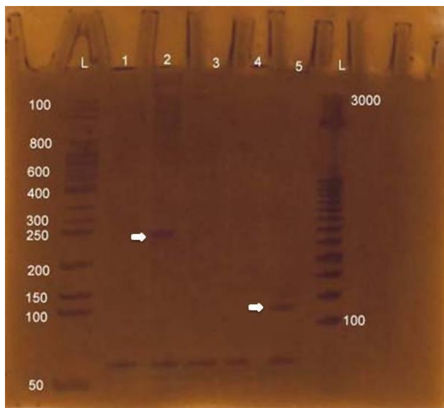
Plate 18. Simple sequence repeat fragments generated from five *Helicoverpa armigera* larval morphotypes occuring on amaranthus (lane 1to lane 5) upon amplification with HaSSR1, HaSSR2, HaSSR3 and HaSSR4 when resolved in PAGE (10%)



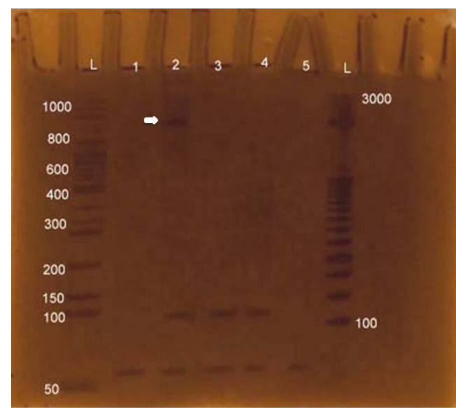
e. HaSSR7



f.HaSSR9



g. HaD47



h. HaC87

Plate 18. Simple sequence repeat fragments generated from five *Helicoverpa armigera* larval morphotypes occuring on amaranthus (lane 1 to lane 5) upon amplification with HaSSR7, HaSSR9, HaD47 and HaC87 when resolved in PAGE (10%)

HaD47 yielded a unique band in both greenish (at 250 bp) and brown with dark dorsal lines (125 bp) and monomorphic at 50 bp in all morphotypes (Plate 18g), whereas HaC87 produced a unique band at 100 bp in both green with dark dorsal lines and brown with orange spots and two unique bands (at 100 bp and 900 bp) in greenish morphotypes. It also produced monomorphic band 50 bp in all morphotypes on amaranthus (Plate 18h).

Finger print of *H. armigera* larval morphotypes occurring on amaranthus based on eight SSR primers was presented in fig. 7. Eight bands produced by all selected SSR primers in all larval morphotypes were represented in green colour code. The bands shared among four, three and two larval morphotypes were indicated by orange, grey and violet colour code, and whereas unique bands present in larval morphotypes were indicated by red colour code.

Among the larval morphotypes occurring on amaranthus, a unique band in green with dark dorsal lines (HaC87), three bands in both brown with orange spots (HaSSR2, HaSSR9, Hac87) and brown with dark dorsal lines (HaSSR1, HaSSR4, HaD47) and nine bands in greenish larval morphotypes, were present.

Genetic variability of *Helicoverpa armigera* larval morphotypes on different crops

Dice coefficient values were calculated based on presence or absence of SSR bands. The calculated value ranged from 0.55 to 1.00 (Table 23). The green with green longitudinal lines (C1) and greenish (C2) larval morphotypes of *H. armigera* occurring on chickpea were found to be most closely related with similarity coefficient of 1.00. Among the larval morphotypes occurring on tomato, green with dark green dorsal lines (T4) and brown with orange spots (T6) shared the most similarity (similarity coefficient of 0.95), whereas least similarity was observed among light green (T1), light green with orange spots (T2) and brown with white lateral lines (T7) (similarity coefficient 0.80). In Okra, brownish (O5) and greenish

Table 23. Dice coefficient matrix for *Helicoverpa armigera* larval morphotypes occurring on tomato, okra, chickpea and amaranthus

	T1	T2	T3	T4	T5	T6	T7	O1
T1	1.0000							
T2	0.8554	1.0000						
T3	0.8313	0.8313	1.0000					
T4	0.8193	0.8916	0.8916	1.0000				
T5	0.8072	0.8313	0.8795	0.9398	1.0000			
T6	0.8675	0.9398	0.8916	0.9518	0.8916	1.0000		
T7	0.8072	0.8072	0.8795	0.9157	0.9036	0.8675	1.0000	
O1	0.6988	0.7711	0.7470	0.8072	0.7711	0.8072	0.7711	1.0000
O2	0.6988	0.7470	0.7229	0.7831	0.7470	0.7831	0.7470	0.9036
O3	0.6627	0.7108	0.6867	0.7470	0.7590	0.7470	0.7108	0.9157
O4	0.6506	0.7470	0.6988	0.7831	0.7470	0.7831	0.7229	0.9277
O5	0.6386	0.7108	0.6867	0.7470	0.7349	0.7470	0.6867	0.8916
O6	0.6506	0.7229	0.6988	0.7590	0.7229	0.7590	0.7229	0.9277
C1	0.5783	0.6265	0.6024	0.6627	0.6024	0.6627	0.6024	0.6867
C2	0.5783	0.6265	0.6024	0.6627	0.6024	0.6627	0.6024	0.6867
C3	0.6627	0.7349	0.6867	0.7470	0.6867	0.7470	0.6867	0.7470
C4	0.6145	0.6627	0.6386	0.6988	0.6386	0.6988	0.6386	0.7229
A1	0.6747	0.7229	0.6988	0.7590	0.7470	0.7590	0.7229	0.9277
A2	0.6386	0.6386	0.6145	0.6747	0.6867	0.6747	0.6386	0.8193
A3	0.6627	0.7108	0.6867	0.7470	0.7349	0.7470	0.7108	0.9157
A4	0.6627	0.7108	0.6867	0.7470	0.7590	0.7470	0.7108	0.8916
A5	0.6988	0.7229	0.7229	0.7590	0.7470	0.7590	0.7229	0.8795

	O2	O3	O4	O5	O6	C1	C2	C3
O2	1.0000							
O3	0.8434	1.0000						
O4	0.8795	0.8916	1.0000					
O5	0.8434	0.9277	0.9398	1.0000				
O6	0.8795	0.8675	0.9036	0.8916	1.0000			
C1	0.6627	0.6506	0.6386	0.6506	0.6627	1.0000		
C2	0.6627	0.6506	0.6386	0.6506	0.6627	1.0000	1.0000	
C3	0.7229	0.7108	0.6988	0.7108	0.7229	0.8675	0.8675	1.0000
C4	0.7229	0.6867	0.6747	0.6867	0.6988	0.7952	0.7952	0.8313
A1	0.8554	0.8675	0.8795	0.8434	0.8795	0.6386	0.6386	0.6988
A2	0.7711	0.7831	0.7952	0.7831	0.7952	0.5542	0.5542	0.6386
A3	0.8434	0.8554	0.8675	0.8313	0.8675	0.6506	0.6506	0.6867
A4	0.8434	0.8554	0.8675	0.8554	0.8675	0.6265	0.6265	0.6867
A5	0.8313	0.8193	0.8554	0.8434	0.8554	0.6627	0.6627	0.6988

	C4	A1	A2	A3	A4	A5
C4	1.0000					
A1	0.6747	1.0000				
A2	0.6145	0.8916	1.0000			
A3	0.6627	0.9880	0.8795	1.0000		
A4	0.6627	0.9639	0.9036	0.9518	1.0000	
A5	0.6747	0.9277	0.8434	0.9398	0.9157	1.0000

(T1-T7: larval morphotypes on tomato, O1-O6: larval morphotypes on okra, C1-C4: larval morphotypes on chickpea, A1-A5: larval morphotypes on amaranthus)

(O4) were the most similar (similarity coefficient 0.90), light green with orange spots (O2), yellowish green (O3) and brownish larval morphotypes (O5) shared the least similarity (similarity coefficient 0.84). Among the morphotypes occurring on amaranthus light green (A1) and green with dark dorsal lines (A3) shared the most similarity (similarity coefficient 0.98) and least similarity was observed between greenish colour morph (A2) and brown with dark dorsal lines (A5) (similarity coefficient 0.84).

Dice coefficient values were utilized to construct dendrogram by un-weighted pair-group method analysis, UPGMA. Dendrogram analysis revealed the occurrence of 3 clusters and 6 sub clusters (Fig 8). The larval morphotypes occurring on chickpea separated out in a single cluster A. While 7 morphotypes of *H. armigera* from tomato and 6 morphotypes from okra were grouped together in a cluster C and 5 morphotypes collected from amaranthus grouped together in a cluster B.

The larval morphotypes on tomato *viz.*, light green with orange spots (T2), greenish (T3), green with dark green dorsal lines (T4), green with black lines and spots (T5), brown with orange spots (T6) and brown with white lateral lines (T7) were found together in sub cluster C2, whereas light green (T1) separated out in sub cluster C1. All morphotypes occurring on okra was grouped together in sub cluster B1 whereas morphotypes occurred on amaranthus were found together in sub cluster B2. Green with green longitudinal lines (C1) and greenish (C2) and green with brown longitudinal lines (C3) morphotypes infesting chickpea were found together in sub cluster A1, whereas brown with brown longitudinal lines (C4) were grouped under sub cluster A2.

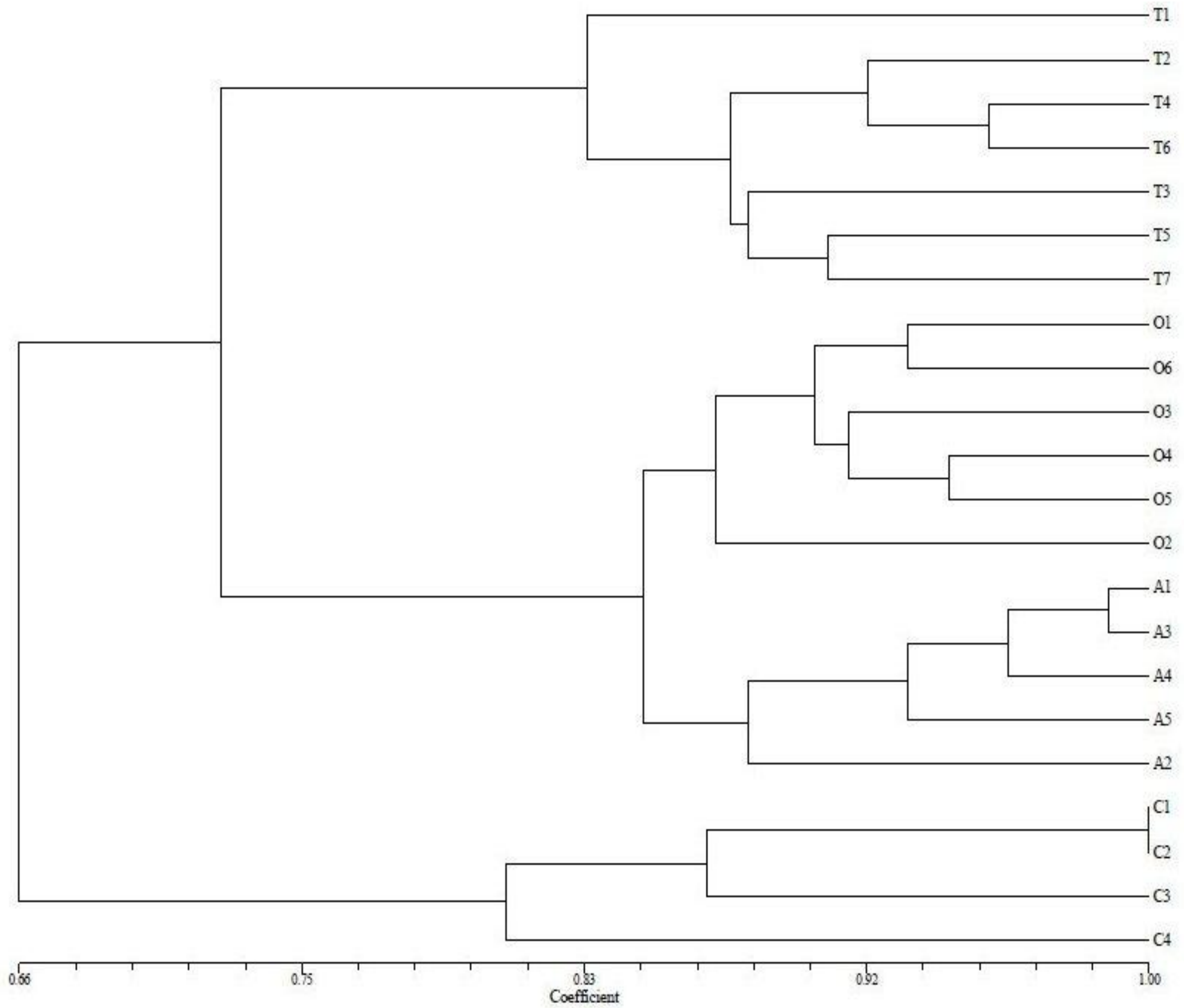


Fig. 8. Dendrogram deduced from matrix of pair wise distances in SSR analysis between twenty two larval morphotypes of *Helicoverpa armigera* using the un-weighted pair-group method analysis, UPGMA.

(T1-T7: Larval morphotypes on tomato, O1-O5: Larval morphotypes on okra, C1-C4: Larval morphotypes on Chickpea, A1-A5: Larval morphotypes on Amaranthus)

4.2.4. Gut metagenomics of *Helicoverpa armigera*

Isolation and quality checking of gut metagenomic DNA from *Helicoverpa armigera*

Metagenomic DNA was isolated from the larval gut of *H. armigera* and confirmed the presence of 16S rRNA fragment in the isolated product by amplification with universal 16S rDNA primers. An intact band at 1500 bp was obtained when resolved at 0.8 per cent agarose gel (Plate 19). The metagenomic DNA was quantified with fluorometer (Qubit 2.0) and the concentration was 309.4 ng/ µl. The hypervariable V3 region of 16S rRNA was amplified with specific primer (Fig. 9) and preceded for 16S rRNA library preparation.

16S rRNA library preparation and sample loading

Metagenomic DNA (5 ng) was taken and standard protocol was followed for 16S rRNA library preparation and sample loading to the Illumina MiSeq™ sequencer.

Illumina sequencing data

Total raw sequencing reads (paired end) of 1,287,940 with average sequence length of 151bp each was obtained from Illumina MiSeq™ sequencer. The following quality parameters were checked. The quality of left and right end of the paired-end read sequences of the sample was shown in the fig. 10. Nearly 90 per cent of the total reads had phred score greater than 30 (>Q30; error-probability >= 0.001).

The base composition distribution of samples were adenine (22.07%), cytosine (27.08%), guanine (29.52%) and thiamine (21.31%) and the average GC content in the range 50-60 per cent was observed in each sequence reads. Application of multiple filters such as conserved region filter, spacer filter and mismatch filter had resulted 1080971, 1079942 and 954500 reads respectively. While making consensus

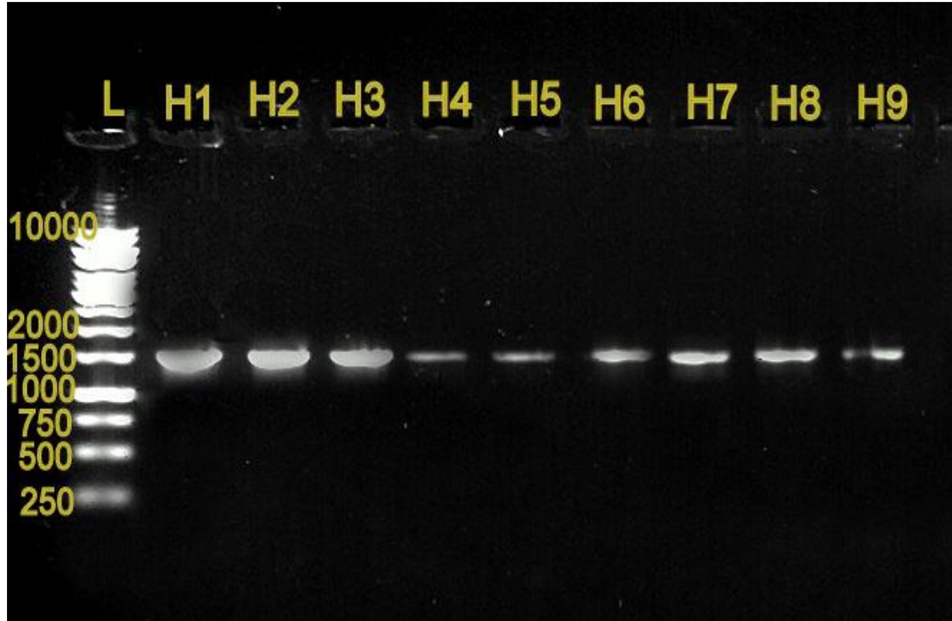


Plate 19. Agarose gel electrophoresis of metagenomic DNA isolated from larval gut of *Helicoverpa armigera* upon amplification with I6S rDNA primer. In the figure, L: 1 kb ladder, H1-H9: *Helicoverpa* larval gut metagenomic DNA from 9 samples

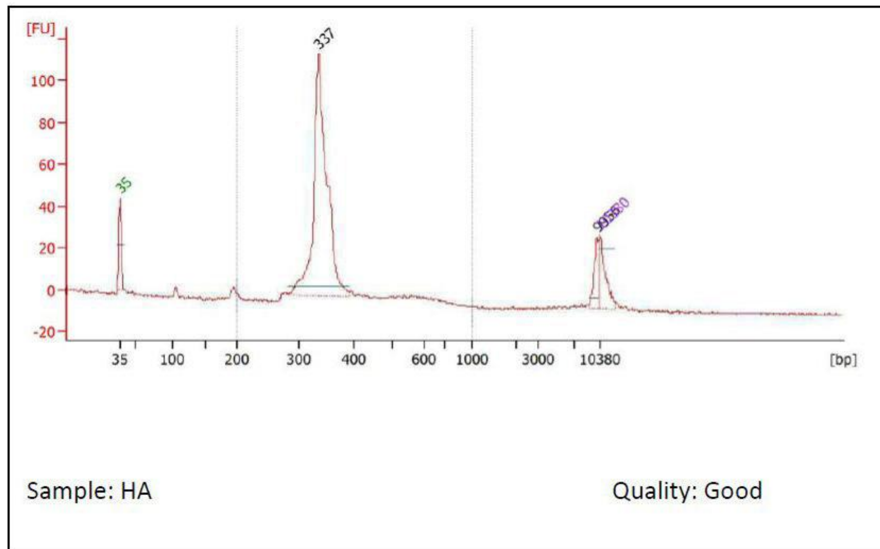
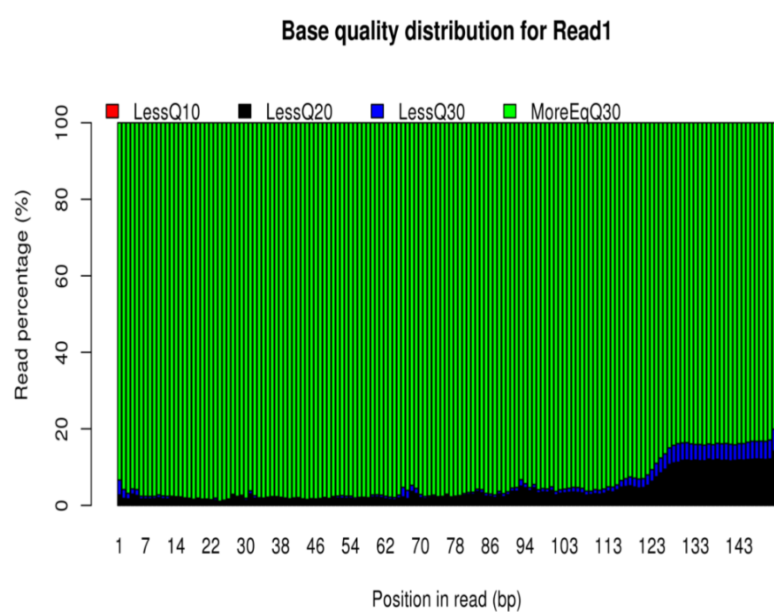


Fig. 9. The amplification of V3 region shown by fluorometer (Qubit 2.0)



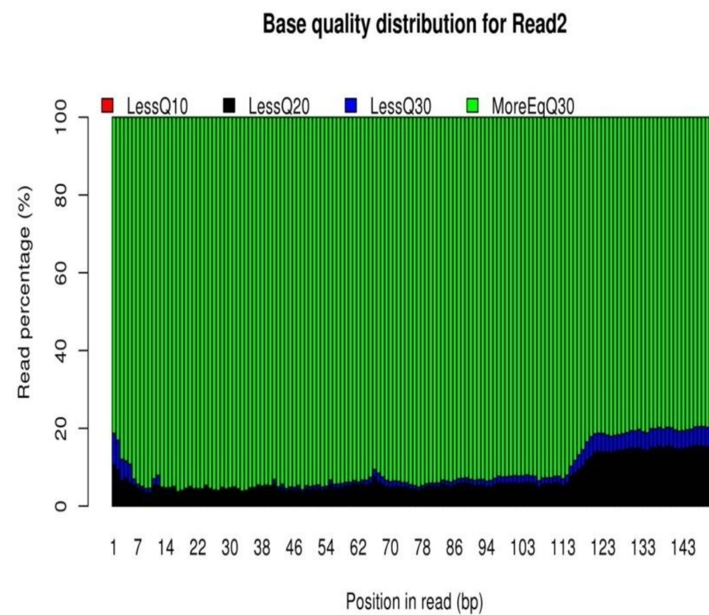


Fig. 10. Base quality distribution of paired end sequences (read 1 and read 2)

V3 sequence, more than 80 per cent of the paired-end reads were aligned to each other with zero mismatches with an average contig length of 135 to 160 bp (Fig. 11).
.

From the 954,500 consensus reads, singletons and chimeric sequences were removed and thus we obtained 864,813 high quality pre-processed reads. The pre-processed reads from all samples were pooled and clustered into OTUs based on their sequence similarity (similarity cutoff = 0.97) and a total of 2303 OTUs were identified from 864, 813reads (Fig. 12). The total number of bacteria species detected in the sample was strongly affected by number of sequence analyzed (Shi *et al*., 2012). We carried out rarefaction analysis to verify the amount of sequencing reflected in the diversity of original microbial community and the analysis revealed that the species count increased sharply before attaining a plateau (Fig. 13). The alpha diversity (47.21) obtained from rarefaction analysis indicated the extent of bacteria species diversity present in the larval gut of *H. armigera*.

Composition of gut bacterial community of *Helicoverpa armigera* larva identified by Illumina sequencing

We analyzed the composition of bacteria present in the larval gut of *H. armigera* and grouped them into each taxonomic category from phyla to species level. The abundance of 10 major bacterial groups in each taxonomic category is given in table 24. Altogether, 17 bacterial phyla were detected in our sample. Among the phyla, *Actinobacteria* was the most dominant which consisted of 53.02 per cent of total bacterial community in the larval gut of *H. armigera*. It was followed by *Proteobacteria* (10.99). Bacteria belongs to *Firmicutes* consisted of 5.28 per cent, followed by *Acidobacteria* (3.79%) and *Bacteriodetes* (1.11%). Reads belongs to *Cyanobacteria, Gemmatimonades, Chloroflexi, Thermotogae, Deinococcus-Thermus* were found to be the other phyla with only a few reads (<1%). Bacteria belong to

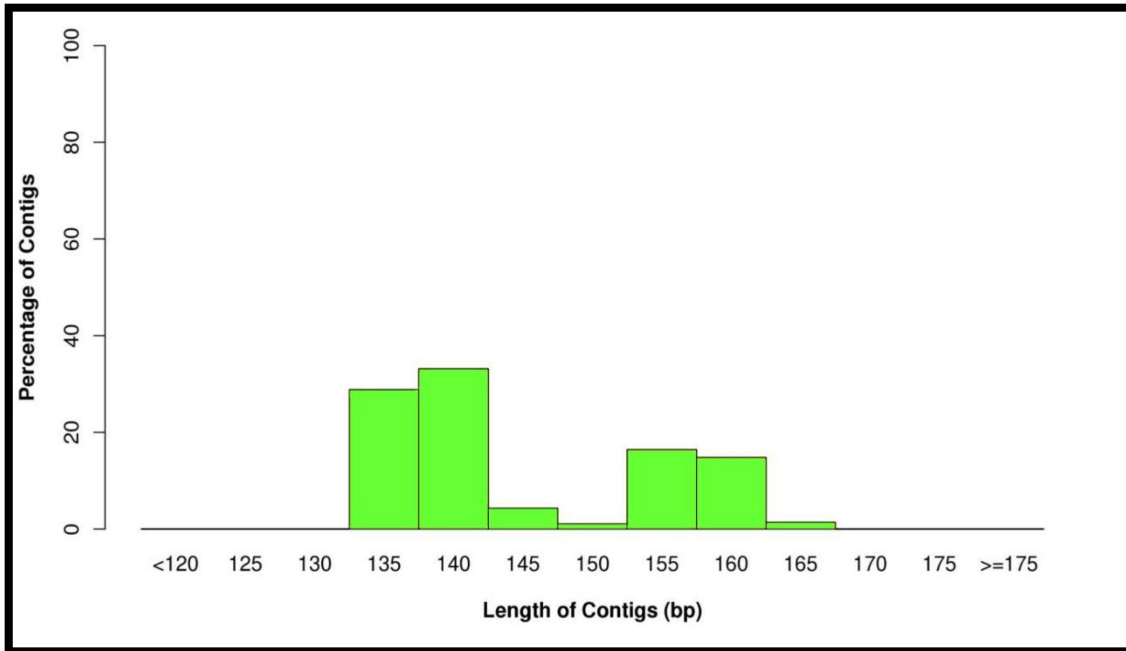


Fig. 11. Contig Length distribution of V3 sequences versus percentage of contigs

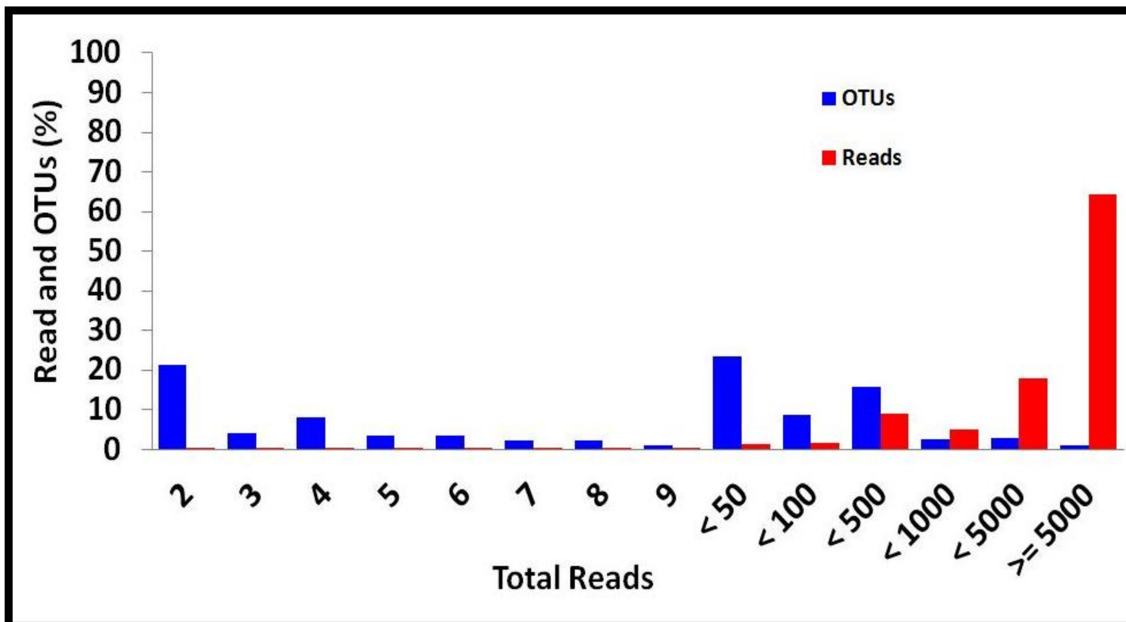


Fig. 12. The percentage of total OTUs and percentage of total read contributed by OTUs

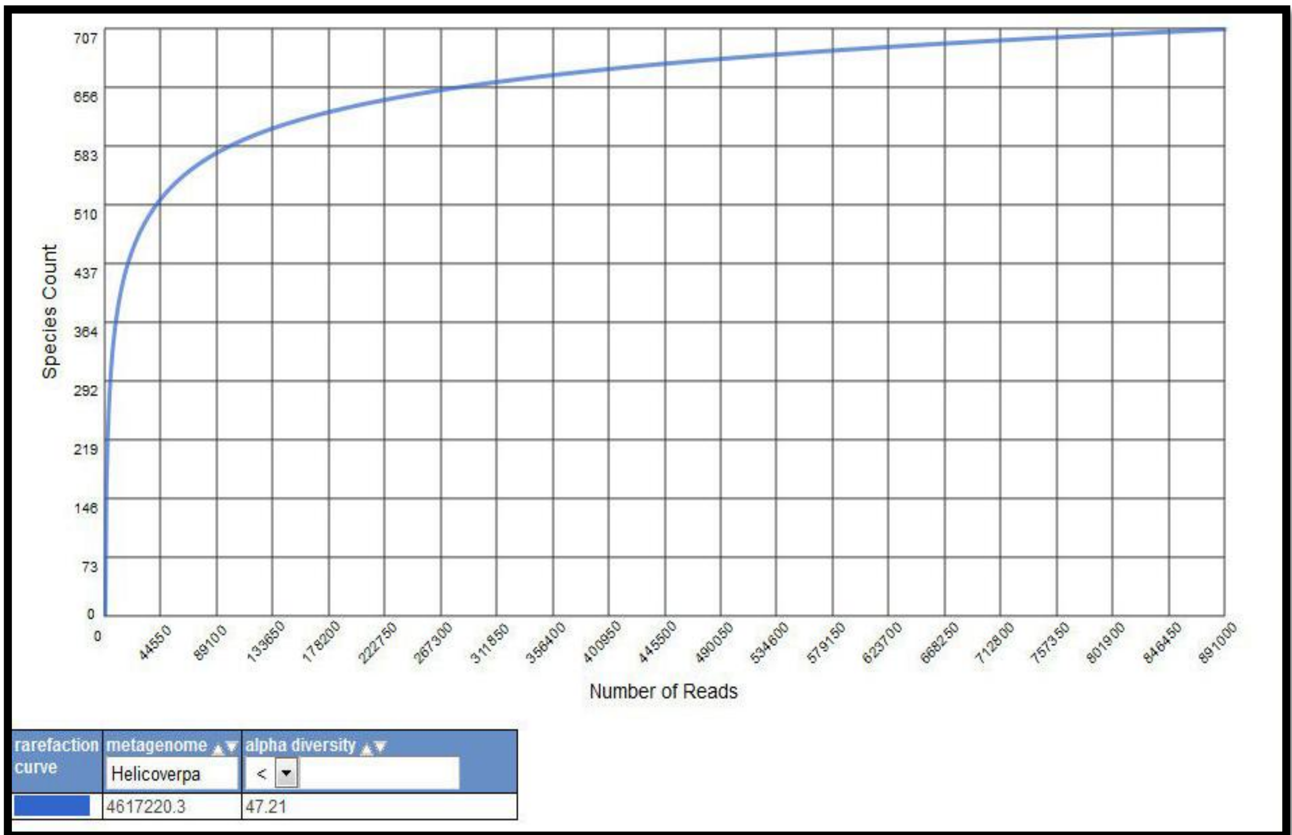


Fig. 13. Rarefaction analyses of *Helicoverpa armigera* gut bacterial communities

phyla *Spirochetes, Tenericutes, Fusobacteria, Verrucomicrobia, Nitrospirae, Fibrobacteres,* and *Aquificae* were also recorded.

A total of 39 bacterial classes were identified and among them *Actinobacteria* [class] was the most dominant group (53.02%) followed by *Gammaproteobacteria* (4.89%), *Alphaproteobacteria* (3.85%), *Clostridia* (2.84%), *Bacilli* (2.36 %), *Deltaproteobacteria* (1.64%), *Acidobacteriia* (0.88%), *Flavobacterii* (0.74%), unclassified (derived from *Cyanobacteria*) (0.60%), *Gemmatimonadetes* (Class) (0.45%). When we analyzed the reads at order level altogether, 84 bacterial orders were detected. The most dominant group was *Actinomycetales* (51.33%), followed by *Alteromonadales* (2.88%), *Clostridiales* (2.72%), *Acidobacteriales* (2.45%), *Bacillales* (2.04%), *Rhizobiales* (1.79%), *Rhodospirillales* (1.40%), *Desulfobacterales* (1.04%), *Thermoleophilales* (0.98%), *Enterobacteriales* (0.86%).

Analyses at family level revealed a total of 173 bacterial families were present in the sample. Major 10 bacterial families present in the sample were *Acidothermaceae* (6.66%), followed by *Pseudonocardiaceae* (5.18%), *Propionibacteriaceae* (4.11%), *Frankiaceae* (3.31%), *Corynebacteriaceae* (2.56%), *Acidobacteriaceae* (2.45%), *Nocardiaceae* (2.27%), *Bradyrhizobiaceae* (1.62%), *Acetobacteraceae* (1.27%), and *Clostridiaceae* (1.25%) (Fig. 13).

Altogether 337 bacterial genera were present. *Acidothermus* (6.58%) was the most dominant group followed by *Propionibacterium* (4.05%), *Corynebacterium* (2.56%), *Acidobacterium* (2.45%), *Arthrobacter* (2.15%), *Saccharopolyspora* (2.14%), *Rhodococcus* (2.04%), *Bradyrhizobium* (1.62%), *Pseudonocardia* (1.44 %), and *Clostridium* (1.18%). When the total reads were analyzed at species level, a total of 707 species were identified in the sample. Analysis at species level showed similarity to the sequences deposited in the RDP database. *Acidothermus cellulolyticus* (6.58%), *Propionibacterium acnes* (3.88%), *Frankia* sp. (3.31%), Shewanella algae (2.75%),

Acidobacterium capsulatum (2.45%), *Arthrobacter ilicis* (2.04%), uncultured *Bradyrhizobium* sp. (1.56%), *Rhodococcus koreensis* (1.32), *Saccharopolyspora* sp. S582 (1.22) and *Thermoleophilum album* (0.98) were the major 10 species identified.

Table 24. Abundance of major 10 taxonomic category from phyla to species level of bacteria occur in the gut of *Helicoverpa armigera*

Sl No	Phylum	Class	Order	Family	Genus	Species
1	*Actinobacteria* (53.02)	*Actinobacteria* (class) (53.02)	*Actinomycetales* (51.33)	*Acidothermaceae* (6.66)	*Acidothermus* (6.58)	*Acidothermus cellulolyticus* (6.58)
2	*Proteobacteria* (10.99)	*Gammaproteobacteria* (4.89)	*Alteromonadales* (2.88)	*Pseudonocardiaceae* (5.18)	*Propionibacterium* (4.05)	*Propionibacterium acnes* (3.88)
3	*Firmicutes* (5.28)	*Alphaproteobacteria* (3.85)	*Clostridiales* (2.72)	*Propionibacteriaceae* (4.11)	*Corynebacterium* (2.56)	*Frankia* sp. (3.31)
4	*Acidobacteria* (3.79)	*Clostridia* (2.84)	*Acidobacteriales* (2.45)	*Frankiaceae* (3.31)	*Acidobacterium* (2.45)	*Shewanella* sp (2.75)
5	*Bacteroidetes* (1.11)	*Bacilli* (2.36)	*Bacillales* (2.04)	*Corynebacteriaceae* (2.56)	*Arthrobacter* (2.15)	*Acidobacterium capsulatum* (2.45)
6	*Cyanobacteria* (0.69)	*Deltaproteobacteria* (1.64)	*Rhizobiales* (1.79)	*Acidobacteriaceae* (2.45)	*Saccharopolyspora* (2.14)	*Arthrobacter ilicis* (2.04)
7	*Gemmatimonadetes* (0.45)	*Acidobacteriia*(0.88)	*Rhodospirillales* (1.40)	*Nocardiaceae* (2.27)	*Rhodococcus* (2.04)	uncultured *Bradyrhizobium* sp. (1.56)
8	*Chloroflexi* (0.44)	*Flavobacteriia* (0.74)	*Desulfobacterales* (1.04)	*Bradyrhizobiaceae* (1.62)	*Bradyrhizobium* (1.62)	*Rhodococcus koreensis* (1.32)
9	*Thermotogae* (0.29)	unclassified(derived from *Cyanobacteria*) (0.60)	*Thermoleophilales* (0.98)	*Acetobacteraceae* (1.27)	*Pseudonocardia* (1.44)	*Saccharopolyspora* sp. S582 (1.22)
10	*Deinococcus-Thermus* (0.13)	*Gemmatimonadetes* (class) (0.45)	*Enterobacteriales* (0.86)	*Clostridiaceae* (1.25)	*Clostridium* (1.18)	*Thermoleophilum album* (0.98)

Proportion [%] of each category is given in parenthesis

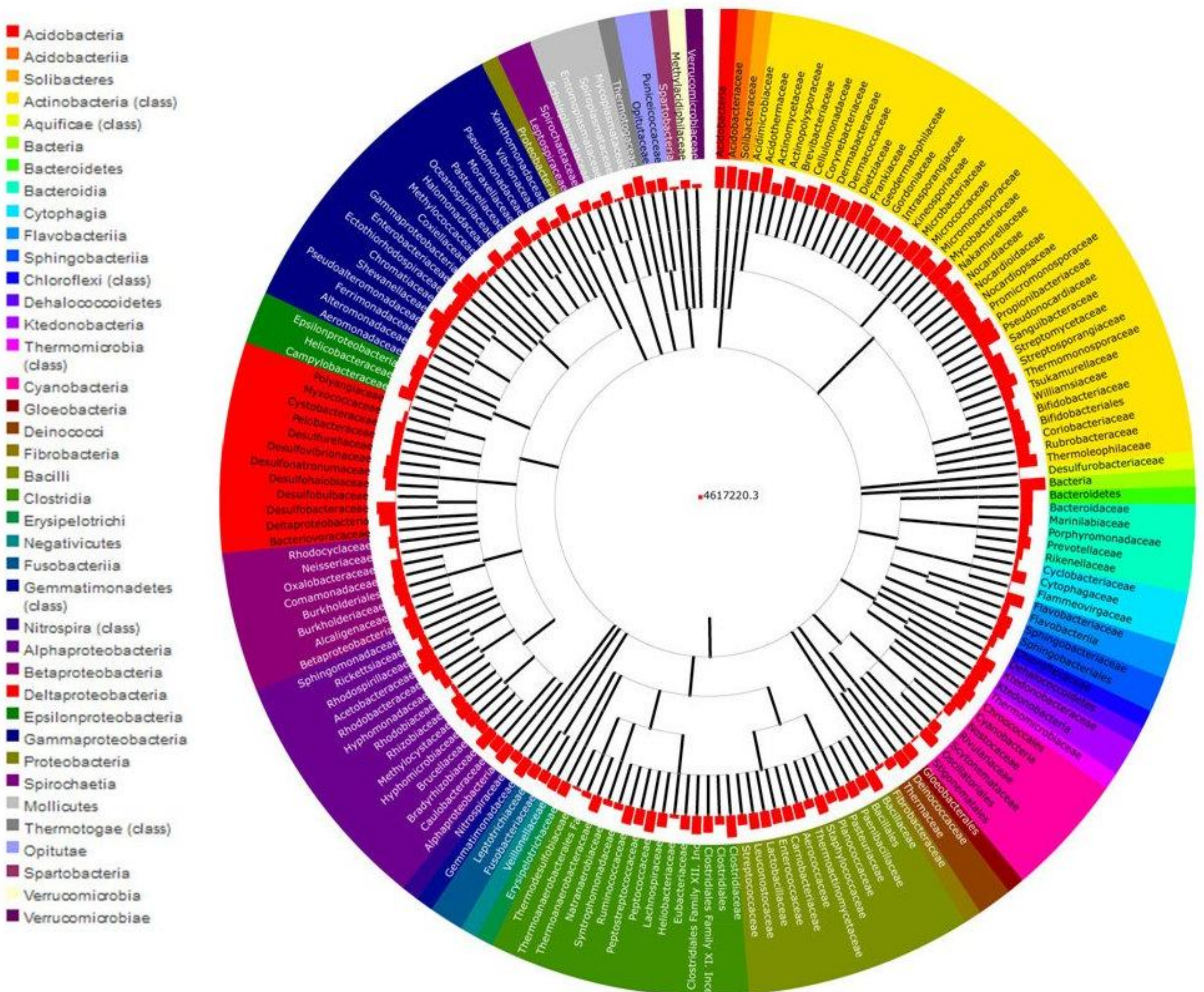


Fig. 14. Phylogenetic tree of bacteria at family level constructed in MG-RAST with illumine sequencing data set (Tree is present with classes (coloured slices) and families belong to each classes are given inside colored slices. The RDP database was used as annotation source, and a minimum identity cutoff [97%] was applied)

Discussion

5. DISCUSSION

The discussion on results obtained from the study on "Identification of larval morphotypes of *Helicoverpa armigera* (Hübner) and their characterization using molecular markers" conducted at Department of Agricultural Entomology, College of Horticulture, Kerala Agricultural University, Vellanikkara are summarized in this chapter to elucidate the various observations and findings.

5.1. Morphological characterization of *Helicoverpa armigera*

In the present study, *H. armigera* larvae collected from four host plants *viz.,* tomato, okra, chickpea and amaranthus belonged to two major colour phases i.e. green and brown. However, variations observed in the shades of colour, markings and longitudinal stripes on larval body which prompted us to categorize them in to different larval morphotypes. Colour forms and the morphometric parameters of active stages of *H. armigera* were studied in detail to mine out differences existing in the population occurring on different crops. Altogether, twenty two larval morphotypes of *H. armigera* were recorded from four host plants under investigation.

5.1.1. Confirming the identity of species

The identification of pests in larval stage is difficult when compared to the adult stage, hence formal keys for the identification of caterpillar heavily depended on chaetotaxy, particularly primary setae (Wagner, 2005). The basic numbers of primary setae on body segment was of lepidopteran larva was11 and have a specific names based on position, it included two dorsal, two subdorsal, three lateral, three sub ventral and one ventral, and sometimes the prothorax bear two additional setae also (Stehr, 1987). However, in the present study altogether 11 primary setae were observed on prothoracic segments, it included two each of dorsal, subdorsal, lateral, subventral and additional setae, one ventral seta and the two lateral setae aligned horizontally with prothoracic spiracle. Our observations were in accordance with Amate *et al*. (1998) who prepared diagnostic key based

on above characters to distinguish *H. armigera* from related species. The position of each seta on prothoracic segment was recorded and that was in consonance with Goel (1987) who described the taxonomy of Noctuidae with special reference to immature stages, whereas according to Sri *et al*. (2010) *H. armigera* larve were greenish in colour with dark coloured longitudinal stripes and had a dark prothorasic shield extended up to the margin of subdorsal seta (SD_1).

The consistency in genital character is universally recognised and as in any other taxonomic study, identification of species by means of genitalia depends upon a valid recognition of morphological characters (Siverly, 1947). The observation made on male genitalia structure was in accordance with previous workers. According to Hardwick (1970) male genitalia of *H. armigera* consisted of long to moderately long valves with broadened apical possed numerous coronas. Whereas, Brambila (2009) distinguished the species of *H.armigera* based on cornuti count. If the count of cornuti sets were equal to or less than 12, it could be *H. armigera*, and if the count of cornuti sets exceeds more than 12 sets, it might probably be *H. zea*. If the aedeagus had no cornuti, or very few, the specimen was probably aberrant and sterile.

The adult female genitalia were dissected out and the various parts were recorded. The observation made are in agreement with Hardwick (1965), who reported that in female genitalia of *H. armigera*, dorsal sclerotization at the base of appendix bursae was restricted and it terminated apically in a normal dilation and lumen surface of appendix bursae clothed with spicules. Whereas, ductus bursae and appendix bursae in female in *H. armigera* were observed for possible variation in adult female moth infesting different host plants. Among the population collected from different crops, significantly the highest length of ductus bursae and appendix bursae was recorded in *H. armigera* population collected from chickpea (Patil *et al*., 2012).

Our observation on morphological characters *viz*., setal arrangement on prothoracic segment of larva and genitalia structure of both male and female adult moths were confirmed for the species level of identity of tomato fruit borer as *H. armigera.*

5.1.2. Morphomerty of larval morphotypes of *Helicoverpa armigera* on different crops

5.1.2.1. Morphometry of larva, pupa and adult of *Helicoverpa armigera* morphotypes on tomato

On tomato, seven larval morphotypes *viz*., light green, light green with orange spots, greenish, green with dark green dorsal lines, green with black lines and spot, brown with orange spots and brown with white lateral lines were observed in the present study (Plate 5). These findings are in agreement with Basavannaeppa and Balikali (2014) who reported six colour morphs on tomato *viz*., dark green, greenish, light green, yellowish brown, brownish and greenish brown. Whereas, Jyothi (1991) reported that three distinct colour morphs *viz*., green, black and brown (intermediate) in equal frequencies on tomato, maize and pigeon pea. However, both Yamasaki *et al.* (2009) and Ramos and Rejesus (1976) reported two major colour forms of *H. armigera* larvae *viz.,* green and brown, when reared on tomato leaves and fruits, respectively. According to Ramos and Rejesus (1976), *H. armigera* feeding on tomato fruits rich in carotenoinds attributed to the higher proportion of brown colour morphs. However, larvae reared on tobacco leaves remained in green phase even though beta-carotene was abundant in leaves. The appearance of green phase was possibly depend on this pigment alone, but probably caused by interaction of different pigments ingested by the larvae. Similarly, we found majority of larvae fed on tomato fruits remained in green phase which might be due to interaction effect of other pigments with carotenoinds ultimately reduced its expression.

Seven larval morphotypes of *H. armigera* were observed in tomato growing areas of Palakkad during 33rd to 41st standard week of 2012 and 35th to 41st standard week of 2013(Table 4 and Table 6), whereas six larval morphotypes except brown with white lateral lines were recorded in Thrissur during 41st to 44th standard week of 2012 (Table 5). However, *H. armigera* population was not observed on tomato in Thrissur during 2013. Though the occurrence of *H.*

armigera on tomato in Palakkad was documented for the first time in the present study, Levin (2004) observed *H. armigera* population in tomato throughout the year during the period of 2002-04 except July to September in Thrissur and the highest population was recorded between October and November. Similarly in the present study, the *H. armigera* population was observed on tomato during the month of October, 2012 in Thrissur and it was not observed during the rest of the year due to unavailability of tomato plants in the crop fields.

Among the larval parameters studied, the highest larval length (25.97± 0.97mm), width (3.70±0.47 mm), weight (303.06±0.47 mg) (Table 4a) and width of head capsule (2.31±0.01 mm) (Table 5a) was observed in greenish morphotype. Whereas, brown morphotype with orange spots had the lowest larval length (20.05±0.76 mm), width (3.17±0.24 mm), weight (214.34±13.44 mg) and width of head capsule (2.11±0.08 mm) (Table 4a and Table 6a). According to Sharma *et al.* (2011) the larval length and width of *H. armigera* reared on tomato in the laboratory were 32.40±0.92 mm and 5.2±0.02 mm respectively. Basavaneppa and Balikali (2014) recorded the larval length, width and weight of *H. armigera* colour morphs occurring on tomato in the range of 20-32 mm, 1.5-2.8 mm and 198-294 mg respectively. Our results showed that the larval parameters varied significantly among larval morphotypes and the highest and lowest larval parameters were observed in greenish and brown morphotype with orange spots respectively. This might be due to differential uptake of nutrients by larvae belonged to each morphotypes. However, DNA fingerprint profile analysis (Fig. 7) revealed the existence of genetic variation in larval morphotypes occurring on tomato and the presence of more number of polymorphic bands in greenish morphotypes than brown morphotype with orange spots. Moreover, three unique bands present in the greenish morphotypes at 400 bp, 450 bp and 800 bp might have contributed for the modification of its feeding behavior. Hence, further investigation at gene level must be carried out to confirm the role of gene at particular loci in modifying the feeding behavior of *H. armigera*.

On tomato, male pupae of light green morphotype had the highest pupal length (15.83±0.61 mm) and width (3.30±0.24 mm), whereas pupal weight was highest in greenish morphotype (227.87±20.26 mg). Green morphotype with dark green dorsal lines had lowest male pupal length (14.28±0.84 mm) and brown with orange spot had the lowest male pupal width (3.05±0.23 mm) and weight (158.15±14.34 mg). Female pupal length (15.86±0.61 mm) and weight (226.75±25.21 mg) was highest in greenish morphotype whereas, light green morphotype had the highest pupal width (3.70±0.62 mm). However, lowest female pupal length (14.42±0.63 mm), width (3.16±0.25 mm) and weight (159.94±15.67 mg) was observed in brown morphotype with orange spot (Table 4a, Table 5a and Table 6a). Our results showed that pupal parameters observed higher in female than male. Similarly, Sharma *et al.* (2011) recorded the higher pupal weight and width in female (6.42±0.54 mm; 138.15±1.80 mg) than male (5.98±0.24 mm; 130.60±2.80 mg) even though pupal length was more in male (22.25±0.94 mm) compared to female (18.20±0.95 mm) when reared on tomato. Liu *et al*. (2004) observed a non significant variation in male and female pupal weight (167.7±5.86 mg; 167.0±5.70 mg) of *H. armigera* when reared on tomato. Positive relationship between female pupal size and weight resulting egg compliment or fecundity found in Lepidoptera (Spurgeon *et al*., 1997; Greenberg *et al*., 2005) corroborate the present findings.

Wing and femur characters of both male and female moths emerged out from each larval morphotypes were considered for phenotypic analysis. In most of the insects female wings were larger than male. In the present study, the adult male moths of greenish morphotype on tomato had the highest forewing length (15.75±0.67 mm), width (6.97±0.45 mm) and length of fore, mid and hind femur (3.42±0.02 mm; 3.81±0.05 mm; 3.25±0.02 mm), whereas adults of brown larva with orange spot had the lowest fore wing length (14.20±0.31 mm), width (6.35±0.59 mm) and length of fore, mid and hind femur (3.33±0.03 mm; 3.71±0.08 mm; 3.15±0.05 mm). Female moths of greenish morphotype had the highest fore wing length (15.79±0.57 mm), width (7.05±0.58 mm) and length of

fore, mid and hind femur (3.43±0.03 mm; 3.84±0.09 mm; 3.24±0.05 mm) however, adults of brown larva with orange had the lowest fore wing length (14.30±0.45 mm), width (6.26±0.64 mm) and length of fore, mid and hind femur (3.40±0.07 mm; 3.74±0.06 mm; 3.17±0.05 mm) (Table 4b, Table 5b and Table 6b). Our findings were in agreement with Sharma *et al*. (2011) who recorded the body length and wing expanse of male moth, 18.42 ± 0.58 mm and 38.30 ± 0.35 mm respectively whereas, female moth had 19.82 ± 0.75 mm body length with 42.15 ± 0.65 mm wing expanse.

5.1.2.2. Morphometry of larva, pupa and adult of *Helicoverpa armigera* morphotypes on okra

Six larval morphotypes *viz*., light green, light green with orange spots, yellowish green, greenish, brownish, brown with dark brown longitudinal lines were observed on okra in Thrissur during the period from 2012 to 2014 (Plate 6). Our findings are in consonance with Patil *et al*. (2012) and Basavanneppa and Balikali (2014) who reported six colour morphs on okra *viz*., dark green, greenish, light green, yellowish brown, brownish and greenish brown. Yamasaki *et al.* (2009) recorded only green and brown colour *H. armigera* larvae when reared on okra fruits, flowers and leaves. The incidence of *H. armigera* was observed on okra in Thrissur from 41st to 47th standard week of 2012, 7th to 10th standard week of 2013 and 4th and 5th standard week of 2014. Similarly Levin (2004) observed *H. armigera* population on okra during the period of 2002-04 in Thrissur and the highest population was recorded during the month of November and February.

On okra, yellowish green morphotype had the highest larval length (28.00± 0.47 mm), width (3.93±0.49 mm), weight (320.76±28.05 mg) and width of head capsule (2.25±0.06mm). Greenish morphotype had the lowest larval length (24.05±0.29 mm), width (3.22±0.25 mm) and weight (237.09±15.71 mm); however, lowest width of head capsule (2.18±0.05 mm) was observed in brownish morphotype (Table 7a and Table 8a). The larval parameters of *H. armigera* morphotypes on okra were recorded in detail in the present study. Similarly, Patil

et al. (2012) observed the mean larval length, width and weight of *H. armigera* on okra *viz.*, 30.45±4.0 mm, 2.64±0.36 mm and 295.3±16.90 mg respectively. Basavanneppa and Balikali (2014) recorded slightly lower mean larval length, width and weight (28.4±4.36 mm, 2.37±0.43 mm and 284.65±19.18 mg) in *H. armigera* colour morphs on okra. However, among the *H. armigera* morphotypes on four host plants, the significantly higher larval parameters were observed in okra. This might be due to differential nutritional value of okra when compared to other host plants *viz.*, tomato, chickpea and amaranthus.

The pupal length (16.37±0.55 mm) and weight (226.64±21.33 mg) was observed highest in yellowish green morphotypes on okra, whereas light green morphotype had the highest pupal width (3.45±0.49 mm). However, pupal length (14.58±0.50 mm), width (3.09±0.25 mm) and weight (165.55±23.09 mg) were lowest in male pupa of brownish morphotype. Female pupa of yellowish green morphotype had the highest length (16.43±0.57 mm) and weight (231.68±26.89 mg), whereas pupal width (3.45±0.43 mm) was highest in green morphotype. However, brownish morphotype had the lowest female pupal length (14.75±0.46 mm), width (3.15±0.24 mm) and weight (175.44±20.33 mg) (Table 7a and Table 8a). Similarly Patil *et al*. (2012) recorded the mean length and weight of male (18.20±2.87 mm; 194.8±23.07 mg) and female pupa (17.20±2.10 mm; 245.3±21.65) of *H. armigera* on okra. Casimero *et al*. (2000) studied the effect of larval diets on survival and development of *H. armigera* and results showed the mean pupal weight of *H. armigera* reared on okra fruit was 248.42±4.24 mg.

On okra, highest fore wing length (16.13±0.74 mm) width (7.21±0.24 mm), length of fore, mid and hind femur (3.41±0.03 mm; 3.83±0.04 mm; 3.23±0.02 mm) was recorded in adult male moth of yellowish green morphotype, while lowest fore wing length (14.31±0.59 mm), width (6.08±0.30 mm), length of fore and hind femur (3.35±0.02 mm; 3.16±0.02 mm) was recorded in male moth of brownish larva, and length of mid femur (3.76±0.05 mm) was observed lowest in adults of both greenish and green with orange spot larva. Adult female moths of yellowish green morphotype had the highest fore wing length (16.19±0.68 mm),

width (7.30±0.25 mm) and length of fore, mid and hind femur (3.42±0.03 mm; 3.84±0.03 mm; 3.24±0.04 mm), whereas brownish morphotype was recorded lowest fore wing length (14.75±0.93 mm), width (6.16±0.25 mm) and length of fore, mid and hind femur (3.37±0.04 mm; 3.78±0.09 mm; 3.17±0.02 mm) (Table 7b and Table 8b). Similarly Patil *et al*. (2012) recorded the mean male and female fore wing length (15.07±1.06 mm; 15.56±1.59 mm), width (6.16±0.30 mm; 6.51±0.54 mm) and length of fore, mid and hind femur of male (2.68±0.26 mm; 3.45±0.35 mm; 2.89±0.29 mm) and female (2.94±0.17 mm; 3.64±0.26 mm; 2.86±0.20 mm) moth of *H. armigera* on okra.

On okra, the morphometric parameters of larva, pupa and adults recorded among six larval morphotypes of *H. armigera* varied similar to larval morphotypes on tomato. The differential uptake of nutrients by larvae belonged to each morphotypes might be resulted the difference in morphometric parameters studied. Further, DNA finger print profile analysis of morphotypes on okra revealed that the unique band was observed in all larval morphotypes on okra at different positions. However, presence of unique band at 220 bp might be contributed for the superiority of parameters in yellowish green morphotype over other morphotypes.

5.1.2.3. Morphometry of larva, pupa and adult of *Helicoverpa armigera* morphotypes on chickpea

In the present study, four larval morphotypes *viz*., green with green longitudinal lines, greenish, green with brown longitudinal lines and brown with brown longitudinal lines were recorded on chickpea (Plate 7) for the first time in Kerala. Though chickpea has not been grown as a sole crop in Kerala, populations of *H. armigera* on chickpea in experimental plots have been subjected to both morphological and molecular studies in order to find out variability existing among them. Similarly, Basavanneppa and Balikali (2014) reported six colour morphs on chickpea *viz*., dark green, greenish, light green, yellowish brown, brownish and greenish brown. The incidence of *H. armigera* morphotypes on chickpea was observed from 49th standard week of 2012 to 1st standard week of

2013 in the present study. Similarly, the incidence of *H. armigera* in chickpea fields of Tamil Nadu Agricultural University farm was commenced during the first week of December and it extended up to last week of February (Ravi *et al*., 2005) due to availability of chickpea plants in the farm.

Among the larval morphotypes on chickpea, green morphotype with green longitudinal lines had the highest larval length (24.30±0.83 mm), width (3.26±0.29 mm), weight (248.39±26.51 mg) and width of head capsule (2.20±0.02 mm), whereas greenish morphotype had the lowest larval length (22.25±1.55 mm), weight (237.01±28.52 mm) and width of head capsule (2.15±0.06 mm) and the lowest larval width (3.19±0.13 mm) was observed in green with brown longitudinal lines (Table 6a). Similarly, Patil *et al*. (2012) recorded the mean larval length, width and weight of *H. armigera* colour morphs on chickpea *viz*., 19.33±3.79 mm; 2.78±2.29 mm and 305.80±52.85 mg respectively. Findings of Basavanneppa and Balikali (2014) showed that the mean larval length, width and weight of *H. armigera* colour morphs on chickpea were 29.85±3.49 mm; 1.97±0.51mm and 323.3±3.82 mg respectively. Recently, Yadav *et al*. (2015) evaluated the host plants suitable for *H. armigera* for growth and development and found that among the different plants, highest larval length and weight (35.60 mm; 526 mg) was observed in chickpea. According to previous workers, among *H. armigera* population collected from different host plants, those grown on chickpea recorded the highest body length and weight and it might be due to more nutrient content and high palatability of chickpea when compared to the other host plants. However, in the present study *H. armigera* collected from chickpea recorded lesser morphometric parameters when compared to those reared on tomato and okra. The late planting of chickpea and the varietal characters might be lead to low preference of *H. armigera* towards chickpea that ultimately reduced its growth parameters.

On chickpea, green with green longitudinal lines had the highest male pupal length (15.22±0.99 mm), width (3.13±0.30 mm) and weight (173.53±21.26 mg). The lowest pupal length (14.08±2.24 mm) and weight (166.03±17.51 mg)

was observed in green with brown longitudinal lines whereas, greenish morphotype had lowest pupal width (3.07±0.53 mm). Green morphotype with green longitudinal lines had the highest female pupal length (15.25±0.44 mm), width (3.20±0.26 mm) and weight (179.19±20.18 mg), whereas lowest female pupal length pupal width and pupal weight was observed in green larva with brown longitudinal lines (14.76±2.22 mm), greenish morphotype (3.17±0.25 mm) and brown morphotype with brown longitudinal lines (170.42±26.59 mg) respectively (Table 9a). Similarly, Patil *et al*. (2012) observed that male pupal length (19.10±1.55 mm) of *H. armigera* was more than that of female pupa (17.80±2.40 mm), in converse pupal weight was recorded higher in female (285.2±15.09 mg) than male (197.2±15.52 mg). Studies of Yadav *et al*. (2015) showed that pupal length and weight of *H. armigera* on chickpea was 17.32 mm and 398 mg respectively.

Highest fore wing length (15.28±0.75 mm), width (6.88±0.52 mm) and length of fore, mid and hind femur (3.41±0.03 mm; 3.82±0.04 mm; 3.19±0.04 mm) was observed in adult male moth of green larva with green longitudinal lines on chickpea. While, adult moths of green with brown longitudinal lines had the lowest fore wing length (14.89±0.82 mm) and length of hind femur (3.15±0.04 mm); however, adult of brown larva with brown longitudinal lines had the lowest fore wing width (6.68±0.90), length of fore and mid femur (3.37±0.03 mm; 3.76±0.04 mm). Female moths of green larva with green longitudinal lines had the highest fore wing length (15.68±0.80 mm), width (6.98±0.48 mm) and length of fore, mid and hind femur (3.41±0.03 mm; 3.81±0.06 mm; 3.20±0.04 mm), whereas adult of green larva with brown longitudinal lines was recorded lowest fore wing length (14.98±0.83 mm) and length of mid and hind femur (3.77±0.05 mm; 3.18±0.09 mm), however adult of brown larva with brown longitudinal lines had the lowest fore wing width (6.78±0.57 mm) and length of fore wing (3.39±0.04 mm) (Table 9b). Similarly Patil *et al*. (2012) recorded the mean male and female fore wing length (16.09±0.80 mm; 15.73±1.29 mm), width (6.73±0.42 mm; 6.58±0.28 mm) and length of fore, mid and hind femur of male (3.20±0.17

mm; 3.80±0.40 mm; 3.12±0.40 mm) and female (3.22±0.31 mm; 3.58±0.23 mm; 3.05±0.26 mm) moth of *H. armigera* on chickpea.

In the present study, it was observed that the morphometric parameters of *H. armigera* varied among four larval morphotypes on chickpea as in other host plants. This might be due to differential uptake of nutrients by larvae belonged to each morphotypes. However, SSR marker analysis revealed that green morphotype with green longitudinal lines and greenish morphotypes had similar DNA fingerprints, even though it showed both highest and lowest larval parameters respectively. However, pupal and adult moth parameters were observed lowest in green with brown longitudinal lines.

5.1.2.4. Morphometry of larva, pupa and adult of *Helicoverpa armigera* morphotypes on amaranthus

In the present study, five larval morphotypes *viz*., light green, greenish, green with dark longitudinal lines, brown with orange spots and brown with dark dorsal lines were observed on amaranthus during May 2013 for the period from 19th to 22nd standard week (Plate 8). Previously Levin *et al*. (2004) observed the heavy incidence of *H. armigera* on amaranthus during the month of November and December @ 2-5 larvae per plant.

On amaranthus, light green morphotype had the highest larval length (23.27±1.05 mm), width (3.27±0.26 mm), weight (228.25±36.40 mg) and width of head capsule (2.21±0.01 mm), whereas the lowest larval length was observed in greenish morphotype (21.50±1.13 mm). Brown morphotype with dark dorsal lines had the lowest larval width (3.08±0.66 mm), weight (205.98±14.09 mg) and width of head capsule (2.18±0.06 mm) (Table 10a).

In amaranthus light green morphotype had the highest male pupal length (15.22±0.78 mm) and width (3.18±0.26 mm), whereas highest pupal weight (172.57±21.21 mg) was observed in brown morphotype with orange spots. However, greenish morphotype had the lowest male pupal length (14.56±0.22 mm), width (3.06±0.17 mm) and weight (163.17±18.78 mg). Light green

morphotype had the highest male pupal length (15.39±0.86 mm), width (3.41±0.26 mm) and weight (179.21±22.13 mg), whereas lowest female pupal length (14.63±3.33 mm), width (3.21±0.25 mm) and weight (164.19±18.8 mg) were recorded in greenish morphotype (Table 7a).

On amaranthus, adult male moths of light green morphotype were recorded highest fore wing length (15.09±0.83 mm), width (6.90±0.49 mm) and length of fore, mid and hind femur (3.41±0.04 mm; 3.81±0.02 mm; 3.20±0.02 mm). Adults of brown larva with dark brown dorsal lines had the lowest fore wing length (14.70±0.57 mm), width (6.40±0.22 mm) and length of mid and hind femur (3.72±0.07 mm; 3.17±0.06 mm), whereas adult of brown larva with orange spots had lowest length of fore femur (3.34±0.40). Female moths of light green morphotype had the highest fore wing length (15.09±0.73 mm), width (6.96±0.41 mm) and length of fore, mid and hind femur (3.42±0.03mm; 3.82±0.03 mm; 3.21±0.08 mm), whereas adult of greenish morphotypes recorded the lowest fore wing length (14.43±0.67 mm) and adult of brown larva with dark dorsal lines had the lowest fore wing width (6.61±0.21 mm) and length of fore, mid and hind femur (3.41±0.02 mm; 3.76±0.04 mm; 3.18±0.04 mm) (Table 10b).

Previously, Levin *et al*. (2004) recorded the incidence of *H. armigera* on amaranthus, per cent loss incurred due to its infestation and occurrence of its natural enemies. However, morphometry of larva, pupa and adult moths of *H. armigera* morphotypes occurring on amaranthus were recorded for the first time in the present study.

5.1.3. Morphometry of *H. armigera* morphotypes reared on tomato fruits and chickpea based semi synthetic diet

Among the seven larval morphotypes of *H. armigera* reared on both tomato fruits and chickpea based semi synthetic diet, the highest larval, pupal and adult parameters were observed in greenish morphotype and the morphometric parameters were lowest in green morphotype with black lines and spots. In the present study, morphometric analysis revealed that the mean larval, pupal and

adult parameters of *H. armigera* reared on tomato fruits were comparatively higher than that of reared on chickpea based semi synthetic diet (Table 11; Table12).

Previously, Casimero *et al*., 2000 studied the effect of eight larval diets *viz*., cotton boll and leaf, okra fruit, soyabean seed, tomato fruit and leaf, corn kernel and artificial diet Insecta LF™ on survival and development of *H. armigera* larva in the laboratory and observed that larvae reared on tomato fruit had mean larval duration and pupal weight of 15.04 days and 277.97 mg respectively. The larvae fed with commercial artificial diet (Insecta LF) had comparatively lesser larval duration (12.75 days) and higher pupal weight (324.67 mg). Similarly, Yamasaki *et al.* (2009) explored the effect of plant parts and artificial diet on development of *H. armigera*. Among the pupal parameters studied, significantly the highest pupal weight recorded in larvae reared on artificial diet (318.8±4.8 mg), moderate on tomato fruit (266.2±7.8 mg) and the lowest in cotton flower (94.5 mg).

In the present study, mean larval, pupal and adult parameters of *H. armigera* reared on tomato fruits were comparatively higher than that of reared on chickpea based semi synthetic diet. The transfer of larvae collected from tomato fruits to artificial diet led to the reduced consumption of food which ultimately reduced the morphometric parameters. However, Amer and Sayid (2014) observed significantly the highest pupal weight in *H. armigera* larva reared on pea (330 mg), followed by artificial diet (325 mg) and okra fruit (295 mg).

5.1.4. Frequency (%) of larval morphotypes in population of *H. armigera* on different crops.

In the present study, different larval morphotypes were encountered on tomato, okra, chickpea and amaranthus. The data revealed that there was a dominance of specific morphotypes among different hosts. It was observed that among the seven morphotypes on tomato, greenish morphotype was the dominant both in Palakkad (28.8%) and Thrissur (42.3%), followed by green morphotype

with black lines and spots (24.5%) in Palakkad and light green morphotype (24.2%) in Thrissur. Brown morphotype with white lateral lines had the least population in Palakkad (2.11%), whereas it was absent in Thrissur during 2012 (Table 13). Similarly, greenish morphotypes were dominant on tomato in Palakkad (30.9%) during 2013, and it was followed by light green (22.7%) Brown morphotype with orange spots was recorded the lowest population (2.42%) (Fig. 15). In the present study the frequency of green morphotypes (94%) was higher than that of brown (6%) when reared on tomato fruits. Our findings were in agreement with Basavanneppa and Balikali (2014) who reported the frequency of six colour morphs of *H. armigera* on tomato *viz.*, dark green (30%), greenish (30%), light green (5%), yellowish brown (15%), brown (10%) and greenish brown (10%). According to Yamasaki *et al.* (2009), the proportion of brownish colour (80%) larva was significantly higher than that of green (20%) when reared on fruits, but it was in converse (85 per cent green larva and 15 per cent brown larva) when reared on tomato leaves.

On okra, light green morphotype was dominant (29.2%) followed by yellowish green (27.3%), whereas brown with dark brown longitudinal lines had the lowest population (2.89%) in Thrissur during 2012-13 (Table 14). During 2013, yellowish green morphotypes were dominant (31.9%), followed by light green (24.9%) and brownish morphotypes were recorded the lowest population (3.80%) (Table 18). The frequency of green morphotypes (84%) was higher than that of brown morphotypes (16%) on okra (Fig. 16). Similarly, Basavanneppa and Balikali (2014) recorded frequency of six colour morphs in okra *viz.*, dark green (25%), greenish (25%), light green (10%), yellowish brown (10%), brown (10%) and greenish brown (20%). According to Yamasaki *et al.* (2009), the proportion of brownish colour (70%) larva was significantly higher than that of green (30%) when reared on okra fruits and the proportion of green colour larvae (90%) was more than brown (10%) when reared on okra leaves.

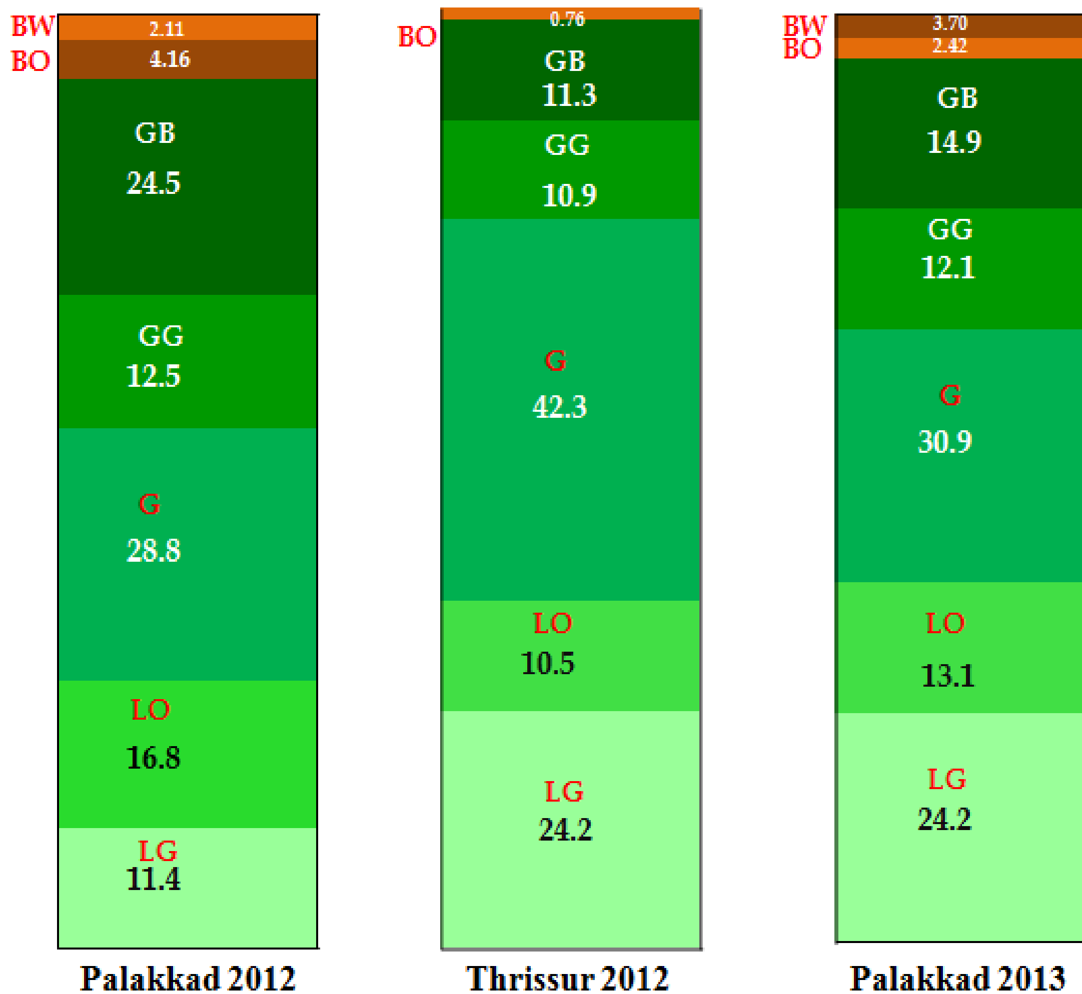


Fig.15. Frequency (%) of larval morphotypes in population of *Helicoverpa armigera* on tomato (LG: light green, LO: light green with orange spots, G; greenish, GG: green with dark green dorsal lines, GB: green with black lines and spots, BO: brown with orange spots, BW: brown with white lateral lines)

and greenish brown (10%). According to Yamasaki *et al*. (2009), the proportion of brownish colour (80%) larva was significantly higher than that of green (20%) when reared on fruits, but it was in converse (85 per cent green larva and 15 per cent brown larva) when reared on tomato leaves.

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Among the four larval morphotypes on chickpea, green with green longitudinal lines was the dominant (41.3%), followed by greenish (26.3%), green with brown longitudinal lines (20.8%) and brown with brown longitudinal lines had the lowest population (11.6%) in 2012 (Fig. 17). In the present study the frequencies of green morphotypes (88%) were recorded higher than brown morphotypes (12%). However, Basavanneppa and Balikali (2014) observed an equal proportion of green and brown colour morphs *viz*., dark green (15%), greenish (20%), light green (15%), yellowish brown (15%), brownish (10%) and greenish brown (25%) on chickpea.

Green morphotype with brown longitudinal lines was the dominant (27.9%) on amaranthus followed by both light green and brown with orange spots (20.7%),

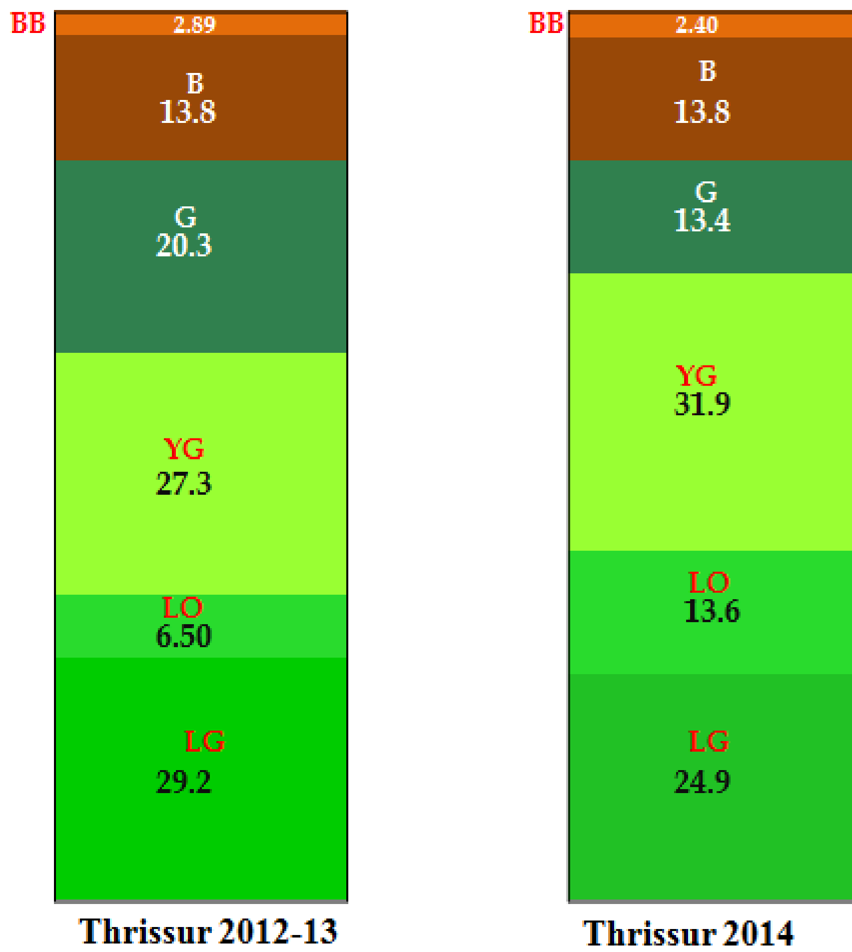


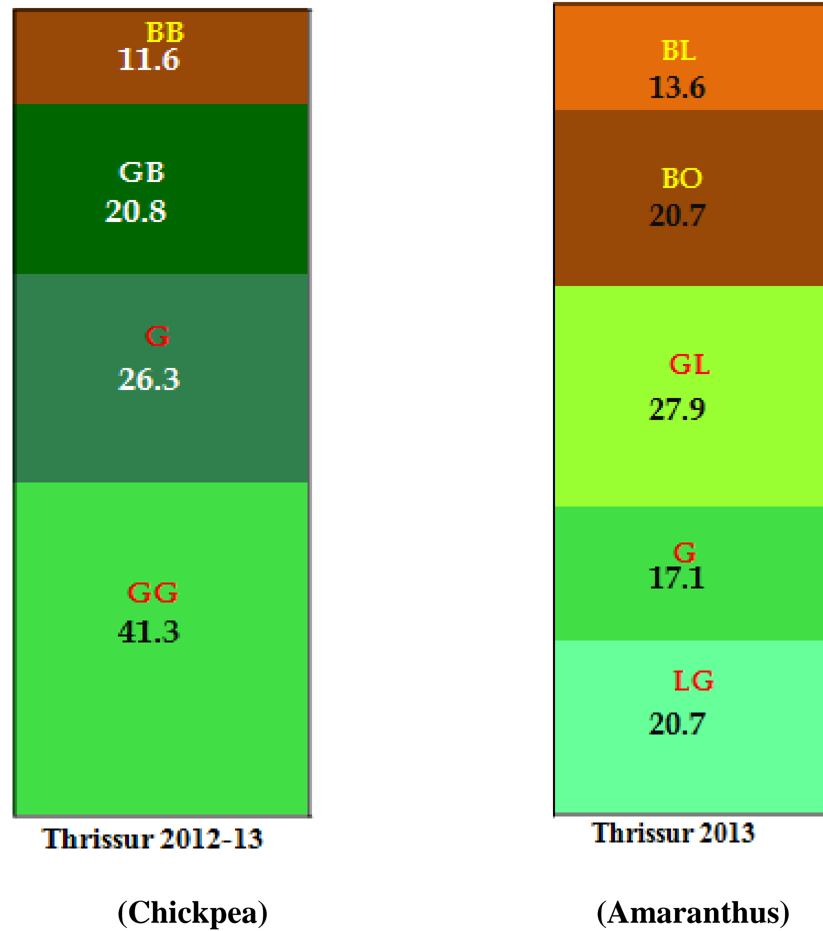
Fig.16. Frequency (%) of larval morphotypes in population of *Helicoverpa armigera* on okra (LG: light green, LO: light green with orange spots, YG; yellowish green, G: greenish, B: brownish, BB: brown with dark brown longitudinal lines)

greenish (17.1%) and the least population was recorded in brown with dark longitudinal lines (13.6%) (Fig. 17).

In the present study it was observed that greenish morphotypes were dominant on tomato when the temperature during the period of incidence ranged from 23 to 31^{0}C. Yellowish green morphotypes were abundant on okra at temperature ranged between 21 and 36.8^{0}C. However, on chickpea green morphotype with green longitudinal lines were dominant at temperature from 21.5 to 30^{0}C and on amaranthus green with dark green longitudinal lines were dominated at mean highest temperature of 23.5-34.4^{0}C. Our findings were in accordance with Hoffmann (1973) who studied environmental control of seasonal variation in the butterfly *Colias eurytheme* and observed the appearance of light coloured butterflies when the atmosphere was hot and dry whereas, deposition of coloured pigments were noticed in butterflies at low temperature. Thus organism responding various ways to changing environmental conditions.

5.1.5. Relative frequency (%) of larva with pigmentation on lateral bands

Lateral banding pattern was considered as the characteristic feature of *H. armigera* larva. The pigmentation pattern on lateral bands *viz.*, continuous, discontinuous or no pigmentation was a highly variable trait among colour morphs collected from different crops. In the present study, major proportion of larvae collected from tomato possessed continuous pigmentation (39.9-66.3%) on lateral bands, followed by discontinuous pigmentation (16.2-25.30%) and larva with no pigmentation (12.1-30.8%) (Table 19a and Table 19c). Similarly, majority of larvae occurring on chickpea (67.5%) and amaranthus (82.9%) possessed continuous pigmentation, which was followed by larvae with no pigmentation (32.5%; 17.1%). However, larvae with discontinuous pigmentation were not recorded on chickpea and amaranthus (Table 19b and Table 19c). On okra, most of the larvae possessed no pigmentation on lateral bands (52.1-56.1%), followed by larva with continuous



(Chickpea) (Amaranthus)

Fig.17. Frequency (%) of larval morphotypes in population of *Helicoverpa armigera* on chickpea (GG: green with green longitudinal lines, G: greenish, GB: green with brown longitudinal lines, BB: brown with brown longitudinal lines) and amaranthus (LG: light green, G: greenish, GL: green with dark longitudinal lines, BO: brown with orange spots, BL: brown with dark longitudinal lines)

pigmentation (27.2-31.7%) and discontinuous pigmentation (16.2-16.7%) (Table 16b and Table 16c). The observations on pigmentation in lateral bands of *H. armigera* occurring on okra and chickpea disagreeing with the findings of Patil *et al.* (2012), who reported that most of the larva collected from okra had the discontinuous pigmentation on lateral bands (54%), followed by continuous (24%) and no pigmentation (22%), whereas most of larva on chickpea possessed no pigmentation (54%), continuous pigmentation (33%) and discontinuous pigmentation (13%). The intensification of colour of longitudinal stripes as well as appearance of appearance of several colour forms might be attributed to the carotenoids present in the plant parts. Further studies should be carried out to confirm biochemical and molecular basis of larval colouration in *H. armigera.*

5.1.6. Intensity of black pigmentation on thorax and last abdominal segments of *H. armigera* larva

In the present study, the intensity of black pigmentation was the highest on last abdominal segments compared to thoracic segments of *H. armigera* larval morphotypes occurring on different crops. On tomato, green morphotype with black lines and spots and brown with white lateral lines had black pigmentation on thorax (1.82 and 1.13) and abdomen (2.52 and 1.57). Brown morphotype with dark brown dorsal lines (1.39 and 1.36) on okra, both green and brown morphotype with brown longitudinal lines on chickpea (1.25 and 1.42; 1.36 and 1.55) and all larval morphotypes noticed on amaranthus except light green larva had black pigmentation on thoracic and last abdominal segments (Table 20). Similar observation was recorded in larva collected from chickpea (1.18 and 1.87) and okra (1.32 and 1.52) with intensity of black pigmentation on thorax and last abdominal segments respectively (Patil *et al*., 2012). The black pigmentation present in larval morphotypes on tomato, chickpea and amaranthus might be depended on temperature (Solensky *et al*., 2003) or biochemical constituents of the host plants (Ramos and Rejesus, 1976), which help in absorption of radiant energy and thus maintain physiological activities in a normal way.

5.1.7. Clustering of larval morphotypes of *H. armigera* based on multivariate analysis technique

The UPGMA dendrogram of cluster analysis based on data of squared Euclidian distances between twenty two larval morphotypes of *H. armigera* revealed three major clusters (Fig. 2). The first cluster contained three larval morphotypes on tomato, two morphotypes on okra, four morphotypes on chickpea and five morphotypes on amaranthus. Second cluster consisted of two morphotypes on okra and third cluster contained four morphotypes on tomato and two morphotypes on okra (at Euclidian distance 8).

At Euclidian distance 25, the twenty two larval morphotypes were grouped into two major clusters. The first cluster consisted of four morphotypes on chickpea and five on amaranthus, three morphotypes on tomato and four morphotypes on okra, whereas second cluster consisted of four morphotypes on tomato and two morphotypes on okra. It was observed that as Euclidian distances increases, the similarity between morphotypes decreases.

Morphometric analysis revealed that six morphotypes (T1, T2, T3, T7, O1 and O3) belonged to second cluster possessed larval, pupal and adult parameters superior over other larval morphotypes and stood together in a separate cluster. Whereas, remaining sixteen morphotypes shared similarity with respect to morphometric parameters and grouped together in first cluster.

Cluster analysis was used to process the insect morphometry data for classifying species based on similar morphometry or classifying certain morphometric characters based on morphometry similarity (Mokosuli, 2013). This technique was successfully employed for morphological discrimination of black legume aphid, *Aphis craccivora* population associated with different host plants (Mehrparvar *et al.*, 2012) and phenotypical morphometric variation of *Aedes aegypti* (Kaunag *et al.*, 2014). In the present study we made a pioneer attempt to cluster *H. armigera* morphotypes occurring on different host plants based on morphometric parameters of larva, pupa and adult moths.

5.2. Molecular characterization of *Helicoverpa armigera* larval morphotypes

5.2.1. DNA barcoding of tomato fruit borer

The DNA barcoding involved DNA sequence analysis of a portion (typically between 600- 900 bp) of the mitochondrial gene cytochrome c oxidase subunit I (COI). In the present study, mtCO1 gene of *Helicoverpa* was used to reveal species identity. The gene sequences were submitted to BOLD and corresponding barcode for *Helicoverpa armigera* was generated (Plate 7). To our knowledge, we have been made the pioneer attempt to barcode *H. armigera* infesting tomato in Kerala.

Genomic DNA was isolated from larval stage of the insect and through barcoding techniques revealed the identity of specimen. The result envisaged the feasibility of using DNA barcode to rapidly assign the unknown specimen at different developmental stages either as a complement to morphological analysis or as the primary diagnostic indicator in cases where the requisite morphological keys are unavailable (Hebert *et al*., 2003).

The barcode of life data base compared the unknown barcode sequence to barcode databases using pairwise sequence divergence calculations (e.g., the Kimura 2-parameter model) as visualized using a neighbor-joining (NJ) tree. Based on the distance model kimura 2 parameter analysis, the nearest neighbourhood of our specimen was from India (Fig. 3). The similar methodology was used effectively in a elucidating the cryptic aphid species in India (Rebijith *et al*., 2013).

Genetic diversity analysis of tomato fruit borer, *H. armigera* based on mitochondrial cytochrome oxidase-I (mtCO-I) also showed that there was no significant variations in the CO-I sequences of *H. armigera* collected on various hosts and geographical locations. However, the phylogenetic tree indicated the possibility of emerging host associated genetic differences in *H. armigera* populations (Asokan *et al*., 2012b).

DNA barcoding had been applied in studying the lepidopteran specimens and correctly assigned them in taxonomic category. The barcode comparisons were successfully applied to distinguish between closely related *Helicoverpa* species, *H. armigera* and *H. zeae* (Behere *et al*. 2007a). Studies also indicated that application of barcoding for species assignment might be taxa-dependent, but with poorly studied or recently diverging groups it became problematic. However, this method had a potential for facilitating the identification of invasive insect pests (Floyd *et al*., 2010). DNA barcoding also supplemented the morphological methods for identifying the invasive armyworm, *Spodoptera* species in Florida (Nagoshi *et al*., 2011).

5.2.2. DNA fingerprint of *Helicoverpa armigera* larval morphotypes

SSR or microsatellites are short DNA sequences of 2-8 base pairs that occur as an array of several hundred copies in the genome of many organisms. They are among the most variable DNA sequences and are used extensively in DNA fingerprinting (Bruford *et al*., 1992). Present work on molecular characterization of *H. armigera* using microsatellite markers led to a pioneer attempt for developing DNA fingerprint profiles and estimating the genetic variability of *H. armigera* larval morphotypes on four different host plants *viz*., tomato, okra, chickpea and amaranthus.

Helicoverpa armigera larval morphotypes on tomato

Fingerprint of *H. armigera* larval morphotypes occurring on tomato was developed based on the presence of clear and distinct bands and molecular weight of bands produced with eight selected SSR primers (Fig. 4). Among the eight primers used in the study, HaSSR2, HaSSR3 and HaSSR4 were highly informative to differentiate larval morphotypes on tomato. Two unique bands were observed in greenish morphotype (at 400 bp and 450 bp) and green morphotype with black lines and spots (150 bp and 210 bp) upon amplification with HaSSR2, whereas HaSSR3 yielded a unique band at 800 bp in greenish

morphotype. HaSSR4 has resulted two unique bands in light green morphotypes (at 150 bp and 200 bp) and three unique bands (at 130bp, 140 bp and 175 bp) in brown morphotype with white lateral lines.

Seven morphotypes of *H. armigera* observed on tomato were distinct with respect to morphological parameters studied. Similarly, a distinct DNA finger print profile was obtained for each *H. armigera* morphotype by SSR analysis. However, three primers used in the study, HaSSR2, HaSSR3 and HaSSR4 had produced unique bands which were able to differentiate four larval morphotypes on tomato *viz*., light green, greenish, green with black lines and spots and brown with white lateral lines.

Previously Subramanian and Mohankumar (2006) demonstrated that the primer HaSSR1 was highly informative to differentiate *H. armigera* population occurring on host plants *viz*., tomato, okra, redgram, blackgram chilli and cotton. Whereas, Khaiban *et al*. (2010) studied the genetic variability of *H. armigera* on tomato in geographic population of West and North West of Iran and found that among the SSR markers screened, HaSSR4, HaSSR6, HaD47 and HaC87 were highly informative for differentiating geographic populations of *H. armigera*.

***Helicoverpa armigera* larval morphotypes on okra**

Out of eight primers used for analysis, six primers *viz*., HaSSR1, HaSSR2, HaSSR7, HaSSR9, HaD47 and HaC87 had produced unique bands in larval morphotypes on okra (Fig. 5). Among them, HaSSR1 produced two unique bands (at 350 bp and 500 bp) in brown morphotype with dark brown longitudinal lines, whereas HaSSR2 yielded a unique band in both yellowish green (at 220 bp) and greenish morphotypes (850 bp). A unique band at 100 bp was observed in brownish morphotype upon amplification with HaSSR7, whereas HaSSR9 yielded a unique band in both light green with orange spots (at 800 bp) and brown morphotype with dark brown longitudinal lines (250 bp). HaD47 had resulted in a unique band in brownish morphotypes (at 100 bp) and two bands in light green

with orange spots (at 450 bp and 800 bp), whereas a unique band at 100 bp was observed in light green morphotypes upon amplification with HaC87.

DNA fingerprint profile analysis of *H. armigera* morphotypes on okra showed that unlike larval morphotypes on tomato, all *H. armigera* morphotypes on okra had been differentiated with six primers used in this study. According to Subramanian and Mohankumar (2006), the primers HaSSR1 and HaSSR4 produced unique bands in *H. armigera* on okra, which differentiated it from population on other crops *viz*., tomato, cotton, chilli, redgram and blackgram.

***Helicoverpa armigera* larval morphotypes on chickpea**

Molecular characterization using eight SSR primers revealed the similar DNA finger print profile in both green with green longitudinal lines and greenish morphotypes, whereas the primers produced unique bands in green morphotype with brown longitudinal line and brown morphotype with brown longitudinal lines (Fig. 6). Four unique bands present in green with brown dorsal lines (HaSSR1, HaSSR4, HaSSR7 and HaD47), whereas nine unique bands present in brown with brown dorsal lines (HaSSR2, HaSSR3, HaSSR7, HaSSR9, HaD47 and HaC87). Unlike morphotypes on other crops, all the primes were able to differentiate two morphotypes on chickpea. To our knowledge, molecular characterization of *H. armigera* on chickpea has been carried out using SSR primers for the first time in the present study.

***Helicoverpa armigera* larval morphotypes on amaranthus**

SSR analysis of genomic DNA revealed the difference in DNA finger print profile of *H. armigera* larval morphotypes on amaranthus (Fig. 7). Seven primers used in the study had produced unique bands and were able to differentiate three morphotypes on amaranthus. Primer HaSSR2 and HaSSR9 produced unique bands in brown with orange spots, whereas HaSSR1, HaSSR4 and HaD47 yielded unique bands in brown with dark dorsal lines. All the primers, except HaSSR3 and HaSSR9 produced unique bands in greenish morphotypes.

Three primers, HaSSR2, HaSSR3 and HaSSR4 were highly informative to differentiate four morphotypes on tomato, whereas six primers had been differentiated all morphotypes on okra. However, all the primers used in this study were able to differentiate two morphotypes on chickpea and seven primers were able to differentiate three morphotypes on amaranthus. Thus DNA fingerprint developed from microsatellite markers could be a valuable tool in identification, monitoring the establishment and dispersal of larval morphotypes of *H. armigera* occurring on different host plants. Perhaps, this has been the pioneer attempt to study the genetic variability of *H. armigera* morphotypes on amaranthus using microsatellite markers.

5.2.3. Genetic variability of *Helicoverpa armigera* larval morphotypes on different crops

In the present study genetic variability of twenty two larval morphotypes of *H. armigera* occurring on four host plants was investigated by PCR analysis of larval genomic DNA using selected eight SSR primers. A total of 83 markers were available for analysis across the different morphotypes. The highest number of 14 markers was produced by both HaSSR1 and HaSSR2, followed by 12 markers generated from HaSSR3. Previously, Khaiban *et al*. (2010) explored the genetic variability of *H. armigera* in tomato growing area of West and North West of Iran using SSR markers. A total of 46 markers from ten SSR primers were used for analysis and the highest number of 14 markers was produced by HaSSR1, followed by eight markers by HaSSR9. Subramanian and Mohankumar (2006) studied genetic variability of *H. armigera* on six crops *viz*., tomato, okra, blackgram, redgram and chilli. A total of 61 markers from ten SSR primers were used for analysis and HaSSR1 produced the highest number of markers (16), followed by HaSSR8 (8) with high degree of polymorphism (75-100%).

Based on the presence or absence of SSR bands, Dice coefficient values were calculated and it ranged from 0.55 to 1.00 (Table 23). The green morphotypes with green longitudinal lines (C1) and greenish (C2) morphotypes

on chickpea were found to be closely related with coefficient value 1.00, whereas morphotypes on chickpea (C1and C2) and amaranthus (A2) was found to be differing widely with coefficient vale of 0.55. The morphotypes on chickpea was distinct from those occur on tomato, okra and amaranthus.

Dice coefficient values were then utilized to cluster the data using UPGMA (Sneath and Sokal, 1973) and the resultant dendrogram (Fig. 8) revealed the existence of three principal clusters and six sub clusters. The larval morphotypes on chickpea stood out in a separate cluster and the morphotypes on tomato, okra and amaranthus shared the similarity and grouped together in a cluster. Within this cluster, seven morphotypes on tomato were grouped together in a principal cluster, C and both the six morphotypes on okra and five morphotypes on amaranthus showed more similarity and grouped together in a cluster, B.

Our results showed that genetic similarity of larval morphotypes on tomato ranged between 81 per cent and 95 per cent, whereas on okra, morphotypes shared genetic similarity of 84 per cent to 95 per cent. *H. armigera* larval morphotypes on amaranthus shared genetic similarity in the range of 84 per cent to 98 per cent, and morphotypes on chickpea shared the similarity between 81 per cent and 100 per cent. The grouping of *H. armigera* larval morphotypes on four crops indicated high similarity among *H. armigera* occurring on vegetable crops *viz*., tomato, okra and amaranthus. However, *H. armigera* morphotypes occurring on chickpea were grouped together in a separate cluster, which shared genetic similarity with larval morphotypes on other three crops in the range of 55 to 74 per cent.

Helicoverpa armigera larval morphotypes on tomato showed genetic similarity with those on okra, chickpea and amaranthus in the range of 65-80 per cent, 57-74 per cent and 63-73 per cent respectively, whereas, morphotypes on okra showed similarity with those on chickpea and amaranthus in the range of 65-72 per cent and 77-91 percent respectively. However, the least similarity was observed in morphotypes occurred on chickpea and amaranthus (55-69 per cent).

The present findings are in agreement with Subramanian and Mohankumar (2006), who studied the genetic variability of *H. armigera* on different host plants using SSR markers and they found that *H. armigera* population on tomato and okra were found to be closely related with genetic coefficient of 0.741. They also observed that *H. armigera* population on cotton and blackgram differed widely with a coefficient of 0.378 and the population on cotton was distantly related with others.

The grouping of *H. armigera* larval morphotypes indicated high similarity among populations collected from vegetable crops *viz*., tomato, okra and amaranthus, while larval morphotypes collected from chickpea crop was found to be variable from other host plants. This phenomenon indicated a strong genetic variability among *H. armigera* morphotypes collected from different host plants. Moreover, Subramanian and Mohankumar (2006) explored the genetic variability among *H. armigera* population collected from different host plants using microsatellite markers. Similarly, Khaiban *et al.* (2010) found that significant genetic differences existed among *H. armigera* on tomato collected from geographical populations of North and North West Iran.

Genetic variability studies on geographically isolated populations of *H. armigera* in India (Fakruddin *et al*., 2004) explained some extent of susceptibility variation among such populations to insecticides (Armes *et al*., 1996) and to microbial insecticides such as *Bacillus thuringiensis* (Gujar *et al*., 2000). Even differential susceptibility has been reported in different colour morphs of *H. armigera* against various chemicals used in redgram ecosystem (Ghante *et al*., 2011). The authors suggest that the differences in *H. armigera* population might be due to variation in plant factors and a host associated genetic difference has been well documented in moth family, Noctuidae (Pashley, 1986). The present study also supported the view that the polyphagous insects tend to be monophagous at micro ecological level (Cunningham *et al*., 1999; Karowe, 1989) as indicated by genetic diversity analysis between larval morphotypes collected from different crops.

According to the previous workers (Ramos and Rejesus. 1976; Yamasaki *et al*., 2009), the larval colouration of *H. armigera* was affected by the host plants. But the occurrence of different larval colourmorphs feeding on same plant parts arised the question of how much variability exists among them. Though genetic control of colouration in *H. armigera* was not known, variability within basic colouration was obvious and a complex set of modifiers must be in operation and that change in quantitative fashion (Jameson and pequegnat, 1974). Bartlet and Raulston (1982) reported that the colour character was under genetic control, whereas Maragal (1990) observed that the green coloured morph readily acquire yellow colour of petals on feeding, while black colour morphs retained melanin to some extent. Furthermore, Mo *et al*. (2005) observed black phenotype mutant strains of adults and pupae in *H. armigera* were pronounced by one recessive gene.

The DNA fingerprinting revealed the existence of unique bands in *H. armigera* population feeding on different crops which might have contributed for altering feeding behavior. Apart from this, the interaction between host plant constituents which the larva feed and the environmental conditions in which it thrives might be responsible for development of morphotypes in *H. armigera*. This aids *H. armigera* for the better survival in different crops ecosystem by highest utilizing the host plant nutrition.

5.2.4. Gut metagenomics of *Helicoverpa armigera*

The feeding behaviour of *H. armigera* was influenced by the gut bacterial communities. So an attempt was made to explore the bacterial communities associated with the gut of *H. armigera*. We isolated gut metagenomic DNA of *H. armigera* by SDS based metagenomic DNA extraction procedure described by Zhou *et al*. (1996) and used the Illumina Next Generation Sequencing platform to reveal total bacterial community present in its gut. Analysis of hypervarible V3 region of 16S rRNA fragment resulted very large bacterial community with 2203 OTUs per sample with 97 per cent identity detection. Previous works showed that

17 culturable bacteria from gut of *H. armigera* based on 16S rRNA sequencing (Madusudan *et al*., 2011). Subsequently bacterial community from midgut of *H. armigera* was isolated by the Cetyl Trimethyl Ammonium Borate (CTAB) based procedure described by Broderick *et al*. (2004) the samples were analysed by capillary electrophoresis in an ABI PRISM DNA sequencer and predicted 29 OTUs per sample (Priya *et al*., 2012).

The bacterial community analysis at Phyla level revealed that *Actinobacteria* was the most dominant group in the larval gut of *H. armigera* followed by *Proteobacteria* and *Firmicutes*. On the contrary, the major bacteria Phyla detected were *Protecobacteria* followed by *Firmicutes* and *Actinobacteria* in the midgut of *H. armigera* larva (Priya *et al*., 2012), gut and reproductive organs of both male and female fruit fly *Bactrocera minax*, gut of ground beetles (Jonathan *et al*., 2007), the Lutzomiya sand fly (Sant'Anna *et al*., 2012) and desert locust, *Schistocerca gregaria* (Dillon *et al*., 2010). However members belonged to *Bacteriodetes* and *Firmicutes* were dominant in gut of termites (Xiang *et al*., 2012) and bees (Mohr and Tebbe, 2006).

Actinobacteria exhibited diverse physiological and metabolic properties, such as the production of extracellular enzymes and the formation of wide variety of secondary metabolites (Schrempf, 2001). *Actinobacteria* associated with termites assisted in nutrient acquisition from a diversity of polysaccharides including cellulose (Pasti and Belli, 1985; Watanebe *et al*., 2003) and hemicelluloses (Schafer *et al*., 1996).Whereas, *Proteobacteria* associated with insects aids in carbohydrate degradation (Delalibera *et al*., 2005), synthesis of B vitamins or essential amino acids (EAA) (Bennet *et al*., 2014) and pesticide detoxification (Werren, 2012). Some members of *Firmicutes* were beneficial to the insects in digestion of cellulose and hemicelluloses (Brown *et al*., 2012). However, entomopathogens viz., *Bacillus thuringiensis* and *Bacillus cereus* were also existed in this group (Raymond *et al*., 2010; Song *et al*., 2014).

Sequence similarity search revealed *Acidothermus cellulolyticus* was the dominant species present in the gut of *H. armigera*. So far this species had not

been isolated and reported from the gut of insects. *Acidothermus* a member of family *Acidothermacea* of Phylum *Actinobacteria* which utilize cellulose and xylan as sole carbon energy sources for growth. It produces E1 endocellulase that are thermally stable and stable over a wide range of pH (Baker *et al*., 1994). Recently the gene encoding for E1 endocellulase has been transformed into rice to produce the enzyme for commercial hydrolysis of cellulose into glucose (Zhang *et al*., 2012). Presence of these bacteria in the larval gut of *H. armigera* might be helpful in effective utilization of various plant parts which are rich in cellulose and xylan.

Propionibacter acnes, another major species detected in the gut of *H. armigera* belonged to family *Propionibacteriaceae*, Phylum *Actiobacteria*. Investigation on bacterial community associated with of *Pentatimids* revealed the occurrence of *P. acnes* in gastric cecae, which are involved in the production of antibiotic barriers against pathogens and supply nutrients to them (Zucchi *et al*., 2012). A nitrogen fixing bacteria, *Frankia* sp (*Actinobacteria*) was also recorded in our sample. These bacteria were found associated with gut of tick, *Ixodes ricinus* and aided them in fixing of atmospheric nitrogen (Carpi *et al*., 2011). *H. armigera* gut harboured *Shewanella* sp. (*Gamma proteobacteria*), which produces beta-glucanase and utilize cellobiose as energy source (Cristobal et al., 2008). Previous work also showed the presence of cellobiose utilizing *Shewanella* sp in the gut of walking stick, *Ramulus artemis* (Shelomi *et al*., 2013).

Arthobacter ilicis (*Actinobacteria*) was found in the larval gut of *H. armigera*.16S rDNA analysis of gut bacterial community in Oriental army worm, *Mythmina seperata* revealed the presence of the *Arthobacter* species in its gut (He e*t al*., 2013). *Arthobacter ilicis* produces the enzyme acetylcholine esterases in marine sponge, *Spirastrella* sp. (Mohapatra and Bapuji, 1998). Occurrences of these bacteria in the gut of *H. armigera* might be helpful in eliciting the binding of synthetic insecticide molecules (oragnoposphorous and carbamates groups) to the enzyme and thus inhibit insecticides on reaching target sites.

Member of *Alpha proteobacteria, Bradyrrhizobium* sp. was associated with gut of *H. armigera.* Analysis on gut of wood feeding passalid beetle *Odentotaenius disjunctus* was also recorded their presence (Navarro *et al*., 2014) however; their functions in insects are yet to be studied. *Rodococcus koreensis*, (*Actinobacteria*) were present in our sample. These bacteria found in the gut of termites (Kurtbke and French, 2007), which are capable of hydrolysing the aliphatic nitriles and benzoyl analogues.

The gut bacterial community analysis of *H. armigera* showed the presence of *Saccharopolyspora* spp. (*Actinobacteria*). These are xylanolytic bacteria which are found associated with gut of *Speculitermes* sp., aids in xylan degradation (Sinma *et al*., 2011). *Thermoleophilum album*s, (*Actinobacteria*) is being reported for the first time in the gut of *H. armigera*. They are able to grow in alkaline substrates and the manganese containing enzymes produced by them are resistant to cyanide inhibition (Perry, 1992) and its presence might be involved in conferring cyanide resistance in *H. armigera*.

The gut bacteria associated with *H. armigera* aids in the breakdown of plant cell wall components *viz*., cellulose, lignocelluloses and xylan. The bacteria detected in *H. armigera* gut such as *Acidobacterium* sp, *Clostridia* sp, *Microbacterium* sp, *Flavobacter johnsoni* and *Thermobia* sp. were involved in digestion of cellulose in other insects (Castaneda and Mallol, 2013; Huang *et al*., 2012; Ngugi et al., 2005; Reid *et al*., 2011; Shil *et al*., 2014). However, *Pseudonocardia* sp and *Clostridia* sp detected in *H. armigera* was responsible for degradation lignocelluloses in wood feeding beetles and termites (Rizzi *et al*., 2013; Lynd *et al*., 2002). Xylanolytic bacteria recorded in termites such as *Serratia* sp. and *Acidobacterium* sp. (Reid *et al*., 2011; Baker *et al*., 1994) are also seen in the gut of *H. armigera*.

Vitamin B producing bacteria *Wigglesworthia* sp detected in the gut of *H. armigera* posses genes encoding for the synthesis of pantothenate (Vitamin B_5), biotin (Vitamin B_7), thiamin (Vitamin B_1), riboflavin FAD (Vitamin B_2), pyridoxine (Vitamin B_6), nicotinamide (Vitamin B_3) and folate (Vitamin B_9) its

genome (Akman *et al*., 2002*). Similarly the* bacterial species detected in *H. armigera viz., Candidatus sp, Buchnera sp, Ishikwanella sp* are capable of supplying essential amino acids to insects thriving on nutrient deficient diets (Baker et al., 1994; Moran and Mira, 2001; Nikoh et al., 2010). Thus the associations of gut bacterial communities' benefitted the *H. armigera* in upgrading the nutrient status of their diet.

Insecticides are widely applying on crops to manage the insect pests and insects develop various mechanisms to tolerate the lethal molecules. The bacteria capable of detoxifying pesticides such as *Burkholderia* were detected in the gut of *H. armigera.* The gut symbionts, *Burkholderia* sp associated with stinkbug use organophosphurus compound fentrothion, as sources of carbon, phosphorus, nitrogen and facilitate detoxification of these compounds (Werren, 2012). Similarly gut bacterium found in *H. armigera, Enterococcus* sp produces cyanide oxygenase and utilize cyanide as a nitrogenous growth substance (Fernandez and Kunz, 2005). *H. armigera* harbours *Rhodococcus* sp. in its gut, which play major role in detoxification of plant defensive compounds *viz.*, phenolics in termite gut (Pasti and Belli, 1985). *Micrococcus* sp. detected in *H. armigera* gut involved in synthesis of antimicrobial peptides which act as defensive compounds against insect pathogens (Bulet *at al*., 1999). Interestingly *H. armigera* also harbours bacteria *viz*., *Bacillus cereus* and *Acarychloris marina* which produce insecticidal toxins (Song *et al*., 2014; Lopez-Pazos and Ceron, 2003) that could be used as weapon against other insect pests.

Our study revealed the composition and diversity of bacterial community associated with tomato fruit borer, *H. armigera* based on Illumina next generation sequencing of 16S rDNA amplicons. Among the bacterial community, the most dominant group were *Actinobacteria*, followerd by *Proteobacteria* and *Firmicutes*. Mining out of functional diversity of bacterial community present in the larval gut revealed their role in making *H. armigera* a successful polyphagous pest of global importance. Our analysis also showed the presence of insecticidal toxin producing bacteria in the gut of *H. armigera*.

Future line of work

- Cross infestation studies of *H. armigera* larval morphotypes among different host plants.
- Find out the genetic variation existing in adult moths of *H. armigera.*
- Study the response of *H. armigera* larval morphotypes to insecticides used in its management.
- Formulate pest management strategy using the genetic variation observed among larval morphotypes.

Summary

6. SUMMARY

Studies on “Identification of larval morphotypes of *Helicoverpa armigera* (Hübner) and their characterization using molecular markers” were conducted at Department of Agricultural Entomology, College of Horticulture during 2012 to 14 and the results of investigation are summarized below.

- Surveys were conducted to find out the incidence of *H. armigera* in vegetable growing areas of Palakkad, Thrissur, Kasaragod and Thiruvananthapuram distinct in Kerala, and flower growing are of Tovalai, Tamil Nadu and the larval samples were collected. *H. armigera* populations collected from tomato in Palakkad and tomato, okra, chickpea and amaranthus in Thrissur were subjected to both morphological and molecular level studies.
- Prothoracic setal arrangement on fifth instar larvae of *H. armigera* was observed and the image was captured using microscope with image analyser software. Altogether 11 primary setae were observed on prothorasic segments, which included two each of dorsal, subdorsal, lateral, subventral and additional setae, one ventral seta and the two lateral setae aligned horizontally with prothorasic spiracle.
- Observations on morphological characters *viz*., setal arrangement on prothoracic segments of larva and adult male and female genitalia structure were confirmed the species level of identity of tomato fruit borer as *H. armigera*.
- Seven larval morphotypes *viz*., light green, light green with orange spots, greenish, green with dark green dorsal lines, green with black lines and spot, brown with orange spots and brown with white lateral lines were observed on tomato in Palakkad, whereas six larval morphotypes (excluding brown with white lateral lines) were observed on tomato in Thrissur.
- Among the larval parameters studied, the highest larval length, width, weight and width of head capsule were observed in greenish morphotype.

The above parameters were lowest in brown morphotype with orange spots.

- On tomato, male pupa of light green morphotype had the highest pupal length and width, whereas pupal weight was highest in greenish morphotype. Green with dark green dorsal lines had lowest male pupal length and brown with orange spot had the lowest male pupal width and weight. Female pupal length and weight was highest in greenish morphotype. Light green morphotype had the highest pupal width. However, lowest female pupal length, width and weight were observed in brown with orange spotted morphotype.
- The adult male moths of greenish morphotype reared on tomato had the highest fore wing length and width and length of fore, mid and hind femur. The above parameters were lowest in adult male moth of brown larva with orange spots. Female moths of greenish morphotype had the highest fore wing length, and length of fore, mid and hind femur. Adults of brown larva with orange spots had the lowest fore wing length, width and length of fore, mid and hind femur.
- Six larval morphotypes *viz*., light green, light green with orange spots, yellowish green, greenish, brownish, brown with dark brown longitudinal lines were observed on okra in Thrissur.
- On okra, yellowish green morphotype had the highest larval length, width, weight and width of head capsule. Greenish morphotype had the lowest larval length, width and weight. Lowest width of head capsule was observed in brownish morphotype.
- The male pupal length and weight was observed highest in yellowish green morphotypes reared on okra, whereas light green morphotype had highest pupal width. Pupal length, width and weight were lowest in male pupa of brownish morphotype. Female pupa of yellowish green morphotype had the highest length and weight, whereas pupal width was highest in light green morphotype. However, brownish morphotype had the lowest female pupal length, width and weight.

- In case of larva reared on okra, highest fore wing length, width and length of fore, mid and hind femur was observed in adult male moths of yellowish green morphotype, whereas lowest fore wing length, width, length of fore and hind femur was recorded in male moths of brownish larvae. But, length of mid femur was observed lowest in adults of both greenish and green with orange spot larvae. Adult female moths of yellowish green morphotype had the highest fore wing length, width and length of fore, mid and hind femur, whereas brownish morphotype was recorded with lowest fore wing length, width and length of fore, mid and hind femur.
- Four larval morphotypes *viz.*, green with green longitudinal lines, greenish, green with brown longitudinal lines and brown with brown longitudinal lines were recorded on chickpea and is being reported for the first time in Kerala.
- Among the larval morphotypes on chickpea, green morphotype with green longitudinal lines had the highest larval length, width, weight and width of head capsule, whereas greenish morphotype had the lowest larval length, weight and width of head capsule and the lowest larval width was observed in green with brown longitudinal lines.
- On chickpea, green with green longitudinal lines had the highest male pupal length, width and weight. Green with brown longitudinal lines was recorded with lowest pupal length and weight and greenish morphotype had lowest pupal width. Green morphotype with green longitudinal lines had the highest female pupal length and width. Lowest female pupal length, width and weight was observed in green larva with brown longitudinal lines, greenish morphotype and brown with brown longitudinal lines respectively.
- Highest fore wing length, width and length of fore, mid and hind femur were observed in adult male moth of green larva with green longitudinal lines on chickpea. Adult moths of green with brown longitudinal lines had the lowest fore wing length and length of hind femur. Adults of brown

larva with brown longitudinal lines had the lowest fore wing width, length of fore and mid femur. Female moths of green larva with green longitudinal lines had the highest fore wing length, width and length of fore, mid and hind femur, whereas adult of green larva with brown longitudinal lines was recorded lowest fore wing length and length of mid and hind femur. Adults of brown larva with brown longitudinal lines had the lowest fore wing width.

- Five larval morphotypes *viz.*, light green, greenish, green with dark longitudinal lines, brown with orange spots and brown with dark dorsal lines were observed on amaranthus.
- Among the *H. armigera* reared on amaranthus, light green morphotype had the highest larval length, width, weight and width of head capsule, whereas, lowest larval length was observed in greenish morphotype. Brown morphotype with dark dorsal lines had the lowest larval width, weight and width of head capsule.
- On amaranthus, adult male moths of light green morphotype have recorded highest fore wing length, width and length of fore, mid and hind femur. Adults of brown larva with dark brown dorsal lines had the lowest fore wing length, width and length of mid and hind femur, whereas adult of brown larva with orange spots had lowest length of fore femur. Female moths of light green morphotype had the highest fore wing length, width and length of fore, mid and hind femur, whereas adult of greenish morphotypes recorded the lowest fore wing length and adult of brown larva with dark dorsal lines had the lowest fore wing width and length of fore, mid and hind femur.
- Among the seven larval morphotypes of *H. armigera* reared on both tomato fruits and chickpea based semi synthetic diet, the highest larval, pupal and adult parameters were observed in greenish morphotype and the morphometric parameters were the lowest in green morphotype with black lines and spots. In the present study, morphometric analysis revealed that the mean larval, pupal and adult parameters of *H. armigera* reared on

tomato fruits were comparatively higher than that of reared on chickpea based semi synthetic diet.

- Frequency (%) of larval morphotypes in population of *H. armigera* revealed that greenish morphotype was dominant in tomato, whereas light green morphotype was abundant in okra. Green morphotype with green longitudinal lines was the dominant on chickpea and Green morphotype with brown longitudinal lines was abundant on amaranthus.
- The pigmentation pattern on lateral bands *viz.*, continuous, discontinuous or no pigmentation was a highly variable trait among morphotypes collected from different crops. We found major proportion of the larvae collected from tomato, chickpea and amaranthus possessed continuous pigmentation on lateral bands, whereas most of the larva collected from okra did not posses pigmentation on lateral bands.
- The intensity of black pigmentation was the highest in last abdominal segments compared to thoracic segments of *H. armigera* larval morphotypes occurring on different crops.
- On tomato, green morphotype with black lines and spots and brown with white lateral lines had black pigmentation on thorax and abdomen. Brown morphotype with dark brown dorsal lines on okra, both green and brown morphotype with brown longitudinal lines on chickpea and all larval morphotypes observed on amaranthus except light green larva had black pigmentation on thoracic and last abdominal segments.
- The UPGMA dendrogram of cluster analysis based on data of squared Euclidian distances between twenty two larval morphotypes of *H. armigera* revealed three major clusters. The first cluster contained three larval morphotypes on tomato, two morphotypes on okra, four morphotypes on chickpea and five morphotypes on amaranthus, whereas second cluster consisted of two morphotypes on okra and third cluster contained four morphotypes on tomato and two morphotypes on okra (at Euclidian distance 8).

- Genomic DNA from twenty two larval morphotypes of *H. armigera* collected from crops *viz*., tomato, okra, chickpea and amaranthus were isolated through modified CTAB method and intact bands were obtained when resolved at 0.8 per cent agarose gel. Spectrophotometric analysis resulted ratio of UV absorbance (A260/A280) between 1.80 and 1.94 indicating the good quality genomic DNA.
- DNA barcoding of *H. armigera* was carried out using the mitochondrial cytochrome c oxidase subunit I (COI) markers. The mtCO1gene sequences were submitted to BOLD and corresponding barcode for *Helicoverpa armigera* was generated.
- DNA extracted from twenty two *H. armigera* larval morphotypes were amplified with eight selected SSR primers, the amplified products were resolved in 10 per cent polyarylamide gel electrophoresis, visualized using silver stain method and photographed.
- Fingerprint of *H. armigera* larval morphotypes occurring on tomato was developed based on the presence of clear and distinct bands and molecular weight of bands produced with eight selected SSR primers. However, three primers used in the study, HaSSR2, HaSSR3 and HaSSR4 had produced unique bands which were able to differentiate four larval morphotypes on tomato *viz*., light green, greenish, green with black lines and spots and brown with white lateral lines.
- DNA fingerprint profile analysis of *H. armigera* morphotypes on okra showed that all six *H. armigera* morphotypes on okra had been differentiated with six primers (HaSSR1, HaSSR2, HaSSR7, HaSSR9, HaD47 and HaC87) used in this study.
- Molecular characterization using eight SSR primers revealed the similar DNA finger print profile in both green with green longitudinal lines and greenish morphotypes, whereas the primers produced unique bands in green morphotype with brown longitudinal line and brown morphotype with brown longitudinal lines. Unlike morphotypes on other crops, all the primers were able to differentiate two morphotypes on chickpea.

- Seven primers used in the study had produced unique bands and were able to differentiate three morphotypes on amaranthus. Primer HaSSR2 and HaSSR9 produced unique bands in brown with orange spots, whereas HaSSR1, HaSSR4 and HaD47 yielded unique bands in brown with dark dorsal lines. All the primers, except HaSSR3 and HaSSR9 produced unique bands in greenish morphotypes.
- In the present study genetic variability of twenty two larval morphotypes of *H. armigera* occurring on four host plants was investigated by PCR analysis of larval genomic DNA using selected eight SSR primers. A total of 83 markers were available for analysis across the different morphotypes. The highest number of 14 markers was produced by both HaSSR1 and HaSSR2, followed by 12 markers generated from HaSSR3 with high degree of polymorphism (75-100%).
- In the present study, genetic similarity of larval morphotypes on tomato ranged between 81 per cent and 95 per cent. Larval morphotypes on okra shared genetic similarity of 84 per cent to 95 per cent and larval morphotypes on amaranthus shared genetic similarity in the range of 84 per cent to 98 per cent. Larval morphotypes on chickpea shared the similarity between 81 per cent and 100 per cent and grouped together in a separate cluster, which shared genetic similarity with larval morphotypes on other three crops in the range of 55 to 74 per cent.
- *Helicoverpa armigera* larval morphotypes on tomato showed genetic similarity with those on okra, chickpea and amaranthus in the range of 65-80 per cent, 57-74 per cent and 63-73 per cent respectively. Larval morphotypes on okra showed similarity with those on chickpea and amaranthus in the range of 65-72 per cent and 77-91 percent respectively. However, the least similarity was observed in morphotypes occurred on chickpea and amaranthus (55-69 per cent).
- The composition and diversity of gut inhabiting bacteria of *H. armigera* was analyzed based on Illumina Next Generation Sequencing of 16S ribosomal RNA amplicons. The data set consisted of 864,813 high quality

paired end sequences with mean length of 150 base pairs. Highly diverse bacterial communities were present in the sample containing approximately 2,303 operational taxonomic units (OTUs).

- A total of 17 bacterial Phyla, 34 Classes, 84 Orders, 173 Families, 334 genera and 707 species were identified from the sequence analysis. *Actinobacteria* was the most dominant groups, followed by *Proteobacteria* and *Firmicutes*.
- The search on function of different gut inhabiting bacteria of *H. armigera* revealed their role in nutrition, detoxification of lethal insecticide molecules and defensive action against pathogens. Insecticidal toxin producing bacterial species were also found associated with the *H. armigera* gut.

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Appendices

Appendix I

Abbreviations and units used

Abbreviations

rpm: rotations per minute

DNA: deoxyribo nucleic acid

RNA: ribo nucleic acid

UV: ultra violet

bp: base pairs

CD: critical difference

MG-RAST: Metagenomic Rapid Annotations using Subsystems Technology

Units

g : gram

mg : milligram

mm: millimetre

% : per cent

^{0}C : degree Celsius

min: minutes

sec: seconds

ng: nanogram

µl: microlitre

Appendix II

Reagents of CTAB buffer

a. CTAB buffer (2X)

-2 per cent CTAB (W/V)

-100 mM Tris-HCl [pH-8]

-10 mM EDTA (pH-8)

-1.5 M NaCl

-2 per cent 2-ß mercaptoethanol

b. Chloroform: isoamyl alcohol (24:1 v/v)

c. 3 M sodium acetate

d. 70 and 95 per cent ethanol `

e. Sterile distilled water

Reagent a. was autoclaved and stored at room temperature

Appendix III

Reagents required for agarose gel electrophoresis

1. Agarose - 0.8 per cent (for genomic DNA)

   - one per cent (for PCR product)

2. 50X TAE buffer (pH8.0)
3. Tracking/loading dye (6X)
4. Ethidium bromide (stock 10 mg/ml; working concentration 0.5 µg/ml)

IDENTIFICATION OF LARVAL MORPHOTYPES OF *Helicoverpa armigera* (Hübner) (LEPIDOPTERA: NOCTUIDAE) AND THEIR CHARACTERISATION USING MOLECULAR MARKERS

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ABSTRACT OF THE THESIS

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Identification of larval morphotypes of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) and their characterization using molecular markers

Abstract

Helicoverpa armigera (Hübner) is a highly destructive, polyphagous pest inflicting sizeable damage in economically important crops worldwide. Notoriety of *H. armigera* is characterized by the ability to thrive in different crop ecosystems, high mobility, fecundity and great capacity to develop resistance to synthetic insecticides used in its management. The presence of larval morphotypes in different crop ecosystems is another feature of this pest. Colour morphs reportedly exhibit differential susceptibility against different insecticides indicating the presence of strong genetic variability with an adaptive significance for the insect. Thus, phenotypic and genetic variability studies could yield valuable information on population structure that can be useful in evolving appropriate strategies for management of genetically distinct morphotypes. A study was therefore envisaged to identify larval morphotypes of *H. armigera* on preferred hosts and to characterize these morphotypes using molecular markers.

Surveys were conducted in vegetable growing areas of Palakkad, Thrissur, Kasaragod and Thiruvananthapuram districts in Kerala, as well as flower growing area of Thovalai, Tamil Nadu during the period between 2012 and 14. The larval populations collected from tomato in Palakkad and also from okra, chickpea, amaranthus and tomato in Thrissur were subjected to morphological as well as molecular level studies. Studies on larval prothoracic setal arrangement, structure of male genitalia and DNA barcoding confirmed the identity of the test insect as *H. armigera*.

Twenty two larval morphotypes were identified from four host plants *viz*., tomato (7), okra (6), amaranthus (5) and chickpea (4) and the frequency of larval morphotypes on each crop were calculated. The dominant morphotypes observed were green on tomato, yellowish green on okra, green with green longitudinal lines on chickpea and green with dark longitudinal lines on amaranthus. Most of the larvae collected from tomato, chickpea and amaranthus possessed continuous pigmentation on lateral bands whereas those collected from okra were without pigmentation on lateral bands. The intensity of black pigmentation on thoracic and last

abdominal segments of morphotypes was worked out based on a standard scale (0-4) and the intensity varied among morphotypes with black pigmentation. The morphometric parameters of larva, pupa and adult were recorded and these showed significant difference among morphotypes on different crops. Further, the morphometric parameters were used for constructing morphocluster based on multivariate analysis techniques and it yielded two major clusters with sixteen morphotypes and six morphotypes in first and second clusters respectively.

Genomic DNA was isolated from twenty two larval morphotypes using modified CTAB method, amplified with selected eight *H. armigera* specific SSR primers, resolved in poly acrylamide gel (10%) and visualized in silver stain (2%). The clear and distinct bands produced were further used for developing DNA fingerprint for individual larval morphotypes. Based on the presence or absence of bands, Dice coefficient of similarity was worked out and the coefficient ranged from 0.55 to 1.00. Dendrogram deduced from similarity coefficient yielded two major clusters and cluster analysis revealed that the morphotypes occurring on vegetables stood together in one cluster, whereas those occurring on chickpea stood out separately in another cluster. Analysis of gut microbiota of larva showed the presence of bacteria belonging to 173 families which helps the insect for its better survival.

The twenty two larval morphotypes observed on four host plants were highly specific with respect to body colour and morphometric parameters. Further, molecular level analysis confirmed the existence of genetic difference among the morphotypes. Hence, present findings suggest that even though *H. armigera* is a polyphagous pest, it could have evolved into different larval morphotypes with great affinity to specific hosts. This could be an adaptive strategy of the pest to reduce intra specific competition and there by ensure better survival on host plants.