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## ABSTRACT

In Senegal, market gardening is one of the most important sectors. It makes it possible to reduce youth unemployment. However, vegetable farming faces several abiotic and biotic problems such as insect pests. The lepidopteran Noctuidae Helicoverpa armigera is one of the most formidable. This study aims to genetically characterize populations of Helicoverpa armigera on its host plants in the Niayes area using the cytochrome oxidase I (COI) gene to see if the host plants have an effect on the genetic structuring of insect populations. Genetic analyses have shown a great haplotypic diversity between the different host plants. A lack of genetic differentiation was also noted between the populations of the different host plants. Similarly, a lack of genetic structuring between the populations of the different host plants (B. oleracea, S. lycopersicum and C. annuum) was noted by a small percentage of variation between the host plants. Demogenetic tests indicate the hypothesis of an expanding population and this hypothesis is confirmed by the sum of the squares of the deviations (SSD) and the irregularity index (Rag) of the populations of H. armigera.

*Keywords:* helicoverpa armigera, host plants, genetic structuring, niayes zone, cytochrome oxidase i..

*Classification:* DDC Code: 576.58 LCC Code: QH455

*Language:* English

London
Journals Press

LJP Copyright ID: 925692
Print ISSN: 2631-8490
Online ISSN: 2631-8504

London Journal of Research in Science: Natural and Formal

Volume 22 | Issue 6 | Compilation 1.0

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## ABSTRACT

*In Senegal, market gardening is one of the most important sectors. It makes it possible to reduce youth unemployment. However, vegetable farming faces several abiotic and biotic problems such as insect pests. The lepidopteran Noctuidae Helicoverpa armigera is one of the most formidable. This study aims to genetically characterize populations of Helicoverpa armigera on its host plants in the Niayes area using the cytochrome oxidase I (COI) gene to see if the host plants have an effect on the genetic structuring of insect populations. Genetic analyses have shown a great haplotypic diversity between the different host plants. A lack of genetic differentiation was also noted between the populations of the different host plants. Similarly, a lack of genetic structuring between the populations of the different host plants (B. oleracea, S. lycopersicum and C. annuum) was noted by a small percentage of variation between the host plants. Demogenetic tests indicate the hypothesis of an expanding population and this hypothesis is confirmed by the sum of the squares of the deviations (SSD) and the irregularity index (Rag) of the populations of H. armigera.*

Keywords: *helicoverpa armigera*, host plants, genetic structuring, niayes zone, cytochrome oxidase i.

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## I. INTRODUCTION

Agriculture is one of the main sectors of activity that contributes to the socio-economic development of populations. It produces most of the basic foodstuffs, improves the livelihoods and incomes of families. In this sector, market gardening occupies a very important place and contributes significantly to food sovereignty [27], to the fight against poverty and family incomes [26, 55]. In West Africa, market gardening is a promising driver of economic growth, a vital source of income for resource-poor producers in rural and peri-urban areas [32]. In Senegal, market gardening is one of the most important sectors. It contributes to the endemic reduction of youth unemployment [45]. However, market gardening faces several abiotic problems (the availability and quality of water for irrigation, hydro-agricultural developments, the low level of technicality as well as the lack of experience of some producers, the lack of qualified supervision, the availability of inputs) and biotic problems such as insects, nematodes and fungi [12, 28]. Many pests have been encountered on vegetable crops. They attack different crops and cause various damages to different parts of the plant [54, 21]. Thus, we were interested in *Helicoverpa armigera*, commonly known as the gram moth or fruit borer, which is a very polyphagous pest whose caterpillars are known to feed on more than 180 host plants belonging to at least 68 plant families [8]. This insect has a large migration capacity of up to 2000 km [5]. Its pupae can enter optional diapause under adverse environmental conditions such as extremely high or low temperatures [17]. With high fecundity and low generation time, capable of generating up to 10 to 11 generations per year [17], *Helicoverpa armigera* is a cosmopolitan pest, which attacks a wide range of hosts: a number of cereal crops (maize, sorghum), legumes such as groundnuts and vegetable crops such as tomatoes (*Solanum lycopersicum* L), cabbage (*Brassica oleracea* L.var capitata), pepper (*Capsicum annuum* L) etc. [12, 19, 37]. The gram moth, *Helicoverpa armigera* (Hubner) is a very formidable insect that colonizes two agro-ecosystems in an asynchronous place [37] and affects the quality and quantity of these crops, source of many economic losses and reduced yield [41, 9, 49]. Its caterpillars can cause very significant damage of up to 85% in Senegal [6], 36% in Nigeria [31]. Thus the management of *H. armigera* has become increasingly difficult due to its high reproductive potential and damage. This is the subject of the highlighting of several methods of struggle by a number of authors in order to eradicate the threats of *H. armigera*. Studies on biology [24, 42], on molecular identification [3], characterization of the mitochondrial DNA COI of *Helicoverpa armigera* [1, 38, 2] etc. have been carried out. However, in Senegal, entomological studies have been carried out in this insect but not genetic studies; hence the importance of our study.

The general objective of this study is to know the genetic diversity and structure of the populations of *Helicoverpa armigera*, a pest of vegetable crops in the Niayes area of Senegal. From this general objective, specific objectives are:

- To determine the genetic diversity of the populations of *Helicoverpa armigera* subservient to the various host plants in the Niayes area (Senegal);
- Characterize the genetic structuring of *Helicoverpa armigera* populations according to these different host plants (*Brassica oleracea, Solanum lycopersicum* and *Capsicum annuum*).

## II. MATERIALS AND METHODS

### *2.1 Presentation of the study area*

This study was carried out in the Niayes area which extends over a length of 180 km bordering the maritime fringe of the North of the country from Dakar to Saint-Louis via the western edge of Thiès and Louga. Its width varies from 05 to 30 km inland [13]. The Niayes are characterized by the alternation of two seasons: a wet season concentrated on three months (July, August and September) and a dry season that lasts the other nine months and during which two cycles of vegetable crops follow one another. A first cycle, from September to January and a second from February to May [12]. The hot and dry climate is characterized by the monsoon that blows during the wet season. Rainfall is low and rarely exceeds 450 mm/year. Temperatures are moderated by the circulation of the sea trade wind and strongly influenced by the cold currents of the Azores [15]. The warmest average monthly temperature hovers around 28°C. Between May and June, the presence of harmattan can raise the temperature to a maximum of 31°C [11].

### *2.2 Sampling*

Our study population consists of a set of individuals from the same host plant. This study was carried out in different localities of the Niayes zone in Senegal: Gorom (Latitude: 14.793060 / Longitude: -17.172220), Keur Abdou Ndoye (Latitude: 14.88762 / Longitude: -17.132322), Malika (Latitude: 14.801484 / Longitude: -17.3376), Mboro (Latitude 15.135985 / Longitude: -16.881218) and Pout (Latitude: 14.771693 / Longitude: -17.059434). In each of these five localities, a significant number of caterpillars of *H. armigera* was taken at random from cabbage (*Brassica oleracea*), tomato (*Solanum lycopersicum*), pepper (*Capsicum annuum*) and lettuce (*Lactuca sativa*). The samples obtained on each host plant are put in a tube containing 95°C alcohol. These samples are coded according to two criteria: the first letter of the genus name in uppercase and the first letter of the name of the species in lower case correspond to the species studied (*Helicoverpa armigera*) and the first letter of the name of the host plant in lower case. Example: samples of *H. armigera* taken from tomatoes are coded by Hat, those taken from cabbage are coded by Hac, those taken from pepper are coded by Hap and finally those taken from lettuce are coded by Hal. These samples obtained are brought back to the laboratory and put in a refrigerator for good preservation.

*Table I:* Summary table of sampling

| Host plants | Sampling codes | Number of individuals sampled |
|---|---|---|
| *Brassica oleracea* | Hac | 13 |
| *Solanum lycopersicum* | Hat | 13 |
| *Capsicum annuum* | Hap | 13 |
| *Lactuca sativa* | Hal | 8 |
| Total | 4 | 47 |

## 2.3 Genetic study

### 2.3.1. *Helicoverpa armigera* genomic DNA extraction

The total DNA of the insects was extracted using the Zymo research tissue protocol. To do this, each caterpillar was placed in a 1.5ml tube in which 95 µl of water and 95 µl of solid Tissue Buffer (Blue) were added for tissue dissociation and cell individualization and then 20 µl of proteinase K was added to degrade all proteins after incubation at 55 ° C throughout the night. The mixture was centrifuged at 12000 rpm for 1 minute to remove tissue debris and recover the supernatant in a new eppendorf tube. 400µl of Genomic Bending Buffer were added to this mixture and then vigorously vortexed. The mixture was then transferred to a Zymo-Spin column previously placed in a collector tube and centrifuged at 12000 rpm for 1 minute. The collector tube was discarded and the Zymo-Spin column was placed in a new collector tube. The DNA, fixed on the column, was then purified to remove any trace of contaminants and to do this, 2 washing pads, capable of passing through the silica membrane after centrifugation, were successively used. First 400 µl of DNA Pre-Wash Buffer were deposited on the Zymo-Spin column and the mixture was centrifuged at 12000 rpm for 1 minute followed by the addition of 700 µl of g-DNA Wash Buffer on the Zymo-Spin column and centrifuged at 12000 rpm for 1 minute. The column was then placed in an eppendorf tube and 50 µl of DNA Elution Buffer was then added directly to the membrane to increase the yield by 15 to 20%. The mixture was then incubated at room temperature for 5 minutes and then centrifuged at 12000 rpm for 1 minute. The DNA thus extracted was stored at -20°C.

### 2.3.2. Electrophoretic migratio

Preparation and deposition of samples electrophoresis consists of separating DNA fragments according to their size by migration into a solid matrix called agarose gel subjected to an electric field. The DNA molecule with a negative charge will migrate under the effect of the electrostatic field to the anode. The distance travelled, measured from the deposition wells, will depend on the size of each fragment. As a result, the larger the size of the fragment, the smaller the distance travelled and vice versa. The samples, 7µl of DNA extracts and 3µl of bromophenol blue (charge blue), will be deposited on a 1.5% agarose gel and migrated at 100 volts for 35 minutes. The migrated DNA is revealed in a darkroom under UV after passing through a bath of Ethidium BET Bromide. The size of the DNA is approximated using a SmartLadder 200bp size marker. The gel is prepared with 1.5 grams of agarose that is added to 100 ml of 0.5X TAE solution.

### 2. 3.3. PCR of cytochrome oxidase I.

#### 2. 3.3.1. Gene choice: Cytochrome oxidase I

Cytochrome oxidase I (COI) is a mitochondrial gene encoding one of the three subunits of cytochrome c oxidase, a transmembrane oxidoreductase enzyme. It is the last to intervene in the mitochondrial respiratory chain. Its length is about 1540 bp [46, 30, 51, 43] in insects; it is widely used in phylogeny and phylogeography. This marker plays a very important role in the barcoding process [10, 33]. Indeed, it is the standard fragment of the genome chosen (at least for the animal kingdom) as a genetic marker for species discrimination and to reveal cryptic diversity [23, 10]. Indeed, its rate of molecular evolution is fast enough to allow the substantial accumulation of mutations (often neutral) and therefore the discrimination not only of very closely related species (twin species) and subspecies, but also the detection of phylogeographic signal within the same species or ecologically differentiated populations (ecotypes) [7, 52, 53, 10]. As a result, it is very decisive in phylogeographic studies [4].

### 2.3.3.2. Amplification

The amplification was carried out in a reaction volume of 25 µl containing for some samples 2 µl of concentrated DNA and for others 3 µl of concentrated DNA with respectively 18,3 µl and 17,3 µl of ultrapure water. For the other constituents, the same volumes were used in both cases namely 2.5 µl of buffer (10x), 1µl of additional $MgCl_2$, 0.2 µl of Taq polymerase, 0.5 µl of dNTP and 0.25 µl of each primer which are 5'GGATCACCTGATATAGCATTCCC3' and 5'CCAGGTAAAATTAAATATAAACTTC3'. It is made by the repetition of cycles which ensures a multiplication by 2 of the target DNA at each cycle.

PCR is performed using the Eppendhorf thermocycler under the following conditions:

- Preliminary denaturation at 94°C for 3 minutes;
- Followed by a repetition of 35 cycles of initial denaturation at 94 °C for 1 minute; hybridization characterized by the attachment of the primers at 48 °C for 1 minute and elongation of the complementary strands at 72 °C for 1 minute;
- Followed by a final extension phase at 72°C for 10 minutes.

The PCR was completed by a hold at 10 °C for the preservation of the product by the thermocycler. An electrophoretic migration on agarose gel was also performed in order to see if the primers have clung.

### 2.3.4. Cytochrome Oxidase I Sequencing

Reactions were performed in a MJ Research PTC-225 Peltier thermocycler with ABIPRISM BigDye TM Terminator Cycle kits. Each sample was sequenced using the sense primer. The fluorescent fragments were purified with the BigDye Xterminator purification protocol. The samples were suspended in distilled water and subjected to electrophoresis in ABI 3730xl sequencer (Appled Biosystems).

### 2.3.5. Genetic Analyses

The sequences were manually corrected and aligned using the Clustal-W algorithm [50] with the BioEdit 5.0.6 software [22]. They have been thoroughly checked, cleaned and aligned to determine the homology of the sites and to be able to perform other phylogenetic analyses including the determination of genetic diversity, genetic differentiation parameters, mutation rates and demogenetic tests. However, after corrections and sequence alignments, we obtained two individuals from lettuce (*Lactuca sativa*) and we eliminated these two individuals because the number of samples from lettuce is too small and can influence genetic structuring since these two individuals can bias sampling.

### 2.3.5.1. Genetic Diversity

The parameters of genetic diversity including the number of polymorphic sites, the total number of mutations, the average number of nucleotide differences, the nature of the mutations (% of transitions and transversions) as well as the haplotypic and nucleotide diversities for each of our study populations were obtained using the DnaSP software version 5.10 [29] and MEGA 5 version v7.0.14 [48].

### 2.3.5.2. Genetic Differentiation and Structuring

The estimation of genetic differentiation between populations generally requires two indices: the degree of genetic differentiation (Fst) and the genetic distance of Nei [34]. The differentiation index (Fst) was evaluated by Harlequin software version 3.5.1.3 [14]. According to Wright, the more Fst tends towards 1, the more genetically structured populations are between them. On the other hand, the populations do not show allelic differences if the Fst is zero. For each value of the Fst, the P-value makes it possible to accept or reject it according to whether it is respectively significant or non-significant. Intra- and inter-population genetic distances were determined with the MEGA5 program version 7.0.14 [48]. Molecular Variance Analysis (AMOVA) was performed to determine the genetic structuring of *H. armigera* populations. The analysis of molecular variance was determined by Harlequin software version 3.5.1.3 [14].

### 2. 3.5.3. Genetic Evolution

Mismatch distribution analysis is the graphical representation of the distribution of genetic distances between individuals in a population. This analysis is accompanied by two indices that test the fit quality of the distribution. These indices are the SSD (sum of squares of deviations) and the Raggedness (irregularity index Rag). The demographic history was estimated using the demogenetic tests, namely the D of Tajima [47] and Fs of Fu [18] under the assumption of neutrality or constant population size. Values close to zero suggest that the population is of constant size while significantly negative or positive values suggest sudden population expansions or bottlenecks respectively. The mistmatch graphs were built with DnaSP software version 5.10 [29]. The SSD and Rag indices and demogenetic tests were obtained with the Harlequin program version 3.5.1.3 [14] and the level of significance was evaluated after 10000 coalescing simulations.

### 2.3.5.4. Phylogenetic Reconstruction

Phylogenetic analysis allows the suggestion of a phylogenetic tree that tries to reconstruct the history of successive divergences during evolution. The haplotype network was built by the Network software ver. 5.0.0.0 in order to identify their relationships as well as graphically explore the existence of possible associations between haplotypes. The phylogenetic reconstructions of *H. armigera* were estimated by four methods: the Neighbour-Joining method is based on the matrix of genetic distances of ecotypes (the Kimura distance 2-parameter) taken two by two to model evolutionary processes; The Maximum Parsimony method considers a tree to be optimal when its total length (number of steps needed to explain the dataset being analyzed) is minimal; The Maximum Likelihood method makes it possible to test all the stories that may have generated the current dataset analyzed and the Bayesian inference which is the process of adapting a probability model to a set of data and summarizes the result by a probability distribution on the parameters of the model and on unnoticed quantities such as predictions for new observations [20].1000000 generations were made by sampling the different parameters every 1000 generations. Generations made during the ignition period are eliminated from the analyses. So conservatively, the first 250,000 generations have been eliminated (25%) and the inferences are then made on the next 750,000 generations. The reliability of a tree is therefore reduced to the reliability of its internal branches (nodes). It is estimated using the MrBAYES ver. program. 3.2.5 [25]. The robustness of the nodes was evaluated for repetitions of 1000 bootstraps. A boostrap is only considered significant if its value is greater than or equal to 70%. The phylogenetic trees of Neighbor-Joining, maximum parsimony and maximum plausibility were built by the MEGA 5 software version 7.0.14 [48] and the Bayesian inference by Mr bayes ver. 3.1.2 [25]. The visualization of the trees was done by the program FigTree version 1.4.2 [39]. The phylogenetic trees of *H. armigera* were rooted by an individual of the species *Helicoverpa zea*.

## III. RESULTS AND DISCUSSION

### 3. 1. Results

### 3.1.1. Genetic polymorphism and diversity

After alignments and corrections of the COI sequences, the dataset obtained consists of 31 sequences, 11 of which come from cabbage (*Brassica oleracea*), 12 from pepper (*Capsicum annuum*) and 8 from tomato (*Solanum lycopersicum*). The dataset comprises 31 sequences each comprising 475 sites including 82 variable or polymorphic sites, 393 invariable sites, 08 singleton sites occupying the positrons: 140; 152; 163; 270; 279; 299; 399; 402 and 74 variable informative sites in parsimonies. The frequency of mutations (Table II) is 49.06% transition type and 50.96% transversion type with 33.25% adenine; 33.25% thymine/uracil; 16.75% cytosine and 16.75% guanine. The mutation rate R is equal to 0.86%.

*Table II:* Percentage of transitions and transversions

| Nucleotides | A | T/U | C | G |
|---|---|---|---|---|
| A | - | *8,47* | *4,27* | **8,22** |
| T/U | *8,47* | - | **8,22** | *4,27* |
| C | 8,47 | **16,31** | - | *4,27* |
| G | **16,31** | *8,47* | *4,27* | - |

The dataset (Table III) includes 31 haplotypes, 11 from *B. oleracea*, 12 from *C. annuum* and 08 from *S. lycopersicum*. The total number of mutations is 80 for individuals of *B. oleracea*; 71 for *C. annuum* and 60 for *S. lycopersicum*. For the species *B. oleracea*, the average number of nucleotide differences is 33,709; that of *C. annuum* is 28.409 and that of *S. lycopersicum* is 27.

*Table III:* Genetic Polymorphism

| Population | Numbers of haplotypes | Total number of mutations, Eta | Mean number of nucleotide differences, k |
|---|---|---|---|
| *Brassica oleracea* | 11 | 80 | 33,709 |
| *Capsicum annuum* | 12 | 71 | 28,409 |
| *Solanum lycopersicum* | 08 | 60 | 27,000 |
| Total | 31 | 88 | 30,301 |

Haplotypic and nucleotide diversities (Table IV) are determined within each individual and for all individuals as a whole. The cytochrome oxidase I (COI) gene reveals high haplotypic diversity and low nucleotide diversity within each population and for all individuals.

*Table IV:* Haplotypic and nucleotide diversity of each population

| Population | Haplotypic diversity (hd) | Nucleotide diversity, Pi(π) |
|---|---|---|
| *Brassica oleracea* | 1,000 ± 0,00150 | 0,07097 ± 0,0000261 |
| *Capsicum annuum* | 1,000 ± 0,00116 | 0,05981 ± 0,0000102 |
| *Solanum lycopersicum* | 1,000 ± 0,00391 | 0,05684 ± 0,0000291 |
| Total population | 1,000 ± 0,00007 | 0,06379 ± 0,0000086 |

### *2.1.2. Genetic differentiation and structuring*

The analysis of genetic differentiation (Table V) shows a value of the degree of genetic differentiation (Fst) negative (-0.02515) and comparable to zero is noted between the population of *C. annuum* and that of *S.*

*lycopersicum*. On the other hand, low Fst values are recorded between the populations of *B. oleracea* and that of *C. annuum*; between *B. oleracea* and *S. lycopersicum*. The Fst also shows non-significant p-value values between the different host plants.

*Table V:* Genetic differentiation between populations

| Populations | *B. oleracea* | *C. annuum* | *S. lycopersicum* |
|---|---|---|---|
| *B. oleracea*<br>P value | *<br>* | | |
| *C. annuum*<br>P-value | 0,03429<br>0,05970 | *<br>* | |
| *S. lycopersicum*<br>P value | 0,02997<br>0,12989 | -0,02515<br>0,91565 | *<br>* |

Intra-population genetic distance (Table VI) is higher in individuals of *B. oleracea* (0.063). On the other hand, it is substantially identical in individuals of *C. annuum* and *S. lycopersicum* (0.03). The genetic distance between the populations of *B. oleracea* and *C. annuum* and between *B. oleracea* and *S. lycopersicum* are identical and equal to 0.051. This genetic distance decreases between populations of *C. annuum* and *S. lycopersicum* (0.033).

*Table VI:* Genetic Distances of Populations

| | Genetic distances | | | |
|---|---|---|---|---|
| | Intra | Inter | | |
| Populations | | *B. oleracea* | *C. annuum* | *S. lycopersicum* |
| *B. oleracea* | 0,063 | | | |
| *C. annuum* | 0,037 | 0,051 | | |
| *S. lycopersicum* | 0,034 | 0,051 | 0,033 | |

Analysis of molecular variance (Table VII) reveals a low value of the variance component, a percentage change (1.69723%) not significant and small (p=0.07624) between host plants. On the other hand, the components of the variance are greater within the host plants and the percentage of variation is significantly high (98.30277%).

*Table VII:* Analysis of Molecular Variance (AMOVA)

| Source of variation | Sums of squares | Variance components | Percentage change |
|---|---|---|---|
| Between host plants | 35,221 | 0,25855 | 1,69723<br>P value = 0,07624 |
| Inside host plants | 419,295 | 14,97484 | 98,30277 |
| Total population | 454,516 | 15,23338 | |

### *2.1.3. Genetic evolution*

The analysis of deogenetic indices (Table VIII) reveals positive D of Tajima values and negative Fs of Fu values. The values of D of Tajima and Fs of Fu have P-values well above 5% so the values of D of Tajima and Fs of Fu are not significant. The values of the demographic indices (Table VIII): the sum of the squares of the deviations (SSD) and Raggedness (Rag) are not significant with P-values well above 5%, so there is a gap between the observed values and those expected, then the population is in demographic expansion.

*Table VIII:* Demogenetic Tests and Demographic Indices

| Host plants Demogenetic tests | *B. oleracea* | *C. annuum* | *S. lycopersicum* | Mean | S.d |
|---|---|---|---|---|---|
| D of Tajima<br>(P-value) | 1,427<br>(0,95) | 1,386<br>(0,95) | 1,231<br>(0,92) | 1,348<br>(0,94) | 0,084<br>(0,01) |
| Fs of Fu<br>(P-value) | -1,227<br>(0,14) | -1,925<br>(0,12) | -0,465<br>(0,26) | -1,206<br>(0,17) | 0,596<br>(0,06) |
| SSD<br>(P-value) | 0,021<br>(0,27) | 0,018<br>(0,25) | 0,022<br>(0,73) | 0,021<br>(0,42) | 0,002<br>(0,22) |
| Raggedness<br>(P-value) | 0,042<br>(0,48) | 0,036<br>(0,35) | 0,043<br>(0,87) | 0,040<br>(0,57) | 0,003<br>(0,22) |

The two Mismatch distribution curves represent the observed (in red) and expected (in green) frequencies which represents the graphical model of an expanding population) as a function of nucleotide differences between sequence pairs.

Analysis of the distribution of genetic distances between individuals of a population taken two to two (Mismatch distribution) shows a multimodal distribution for the three host plants.

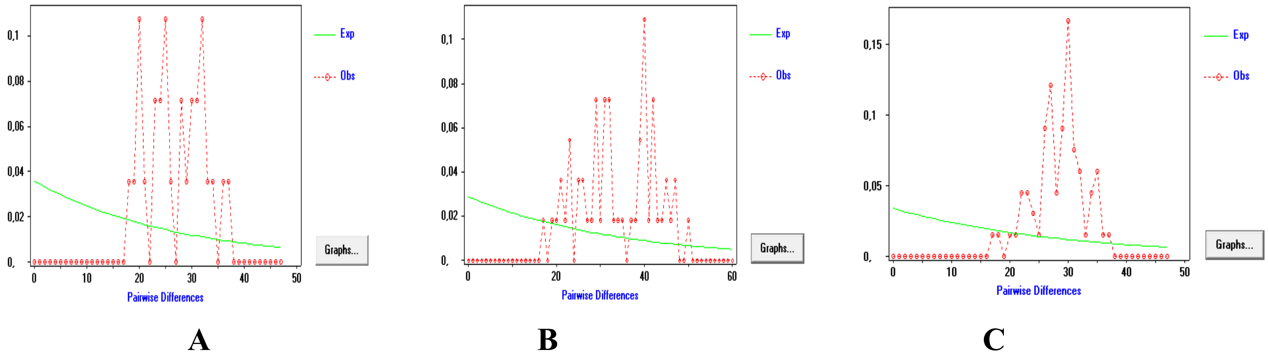


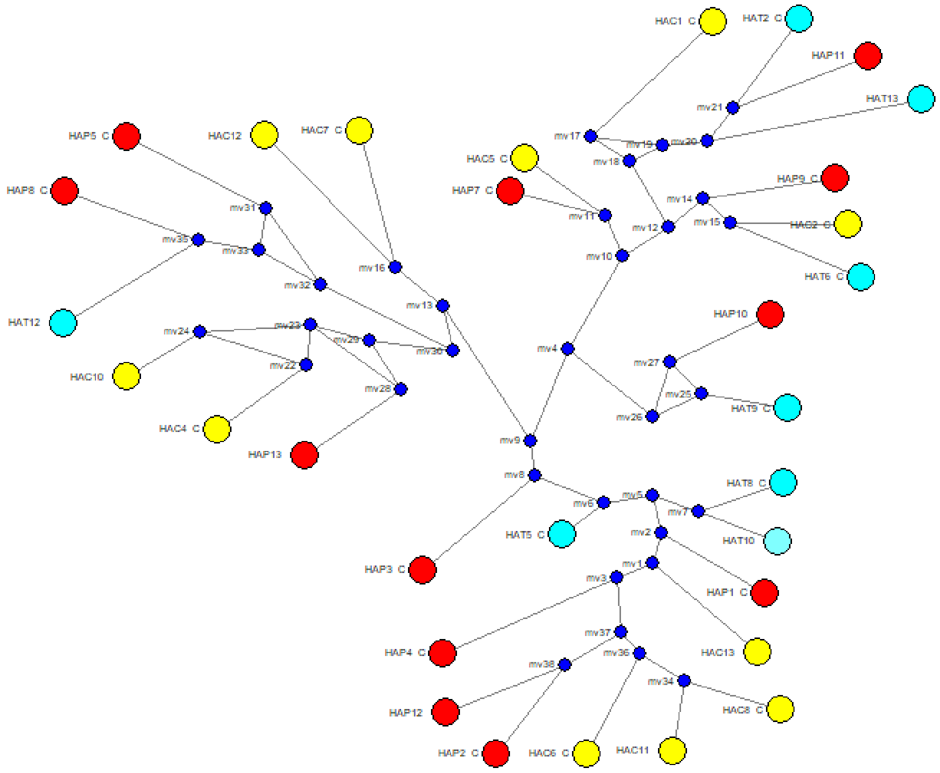
*Figure 1:* Mismatch curves Distribution

Legend: A: Population of *S. lycopersicum*; B: Population of *C. annuum*; C: *B. oleracea* population

### *3.1.4. Phylogenetic reconstructions*

#### *3.1.4.1. Haplotype network*

The dataset is composed of 31 sequences divided into 31 haplotypes that are 99 or even 100% identical to the sequences recorded in the NCBI GenBank database. The haplotype network makes it possible to see the relationships that exist between the haplotypes of the different host plants. Red dots are vectors, i.e. hypothetic haplotypes. The haplotype network shows that all haplotypes are individualized and separated by vectors. The network also shows the presence of three groups of haplotypes and that each group is formed by individuals subservient to the different host plants.



**Legend**

HaP : ● *H. armigera* from pepper

HaC : ● *H. armigera* from cabbage

HaT : ● *H. armigera* from tomato

● Vector

*Figure 2:* Haplotype network of *H. armigera* populations

### *3.2.4.2. Phylogenetic Trees*

Phylogenetic trees were constructed by the methods of Neighbor-joining, Maximum Parsimony, Maximum Likelihood and Bayesian inference. The phylogenetic tree of Neighbor-joining (Figure 3) shows the existence of two clades C1 and C2 and an unsolved Hac8 individual, i.e. one that is not supported by a boostrap value. The first clade C1 shows the presence of individuals subservient to these three host plants (*B. oleracea*, *S. lycopersicum* and *C. annuum*) which are supported by a high boostrap value (92); while in the second clade C2, only two individuals subservient to the population of *B. oleracea* are observed with a boostrap value equal to 75. By the maximum Parsimony method (Figure 4), the tree follows the same topology as that constructed with the Neighbor-Joining method. The phylogenetic tree of maximum likelihood (Figure 5) also shows the existence of two clades C1 and C2. The C1 clade also shows the presence of individuals subservient to these three host plants that are supported by a high boostrap value (94). The second C2 clade only shows the presence of individuals from *Brassica oleracea* that are supported by a boostrap value equal to 61. This highlights that at the level of *B. oleracea* there is a subpopulation that differs from the others. Bayesian inference (Figure 6) shows the presence of a single clade that follows the same topology as the C1 clades recorded in the other methods and that the hac6, hac8 and Hac11 individuals are not resolved.

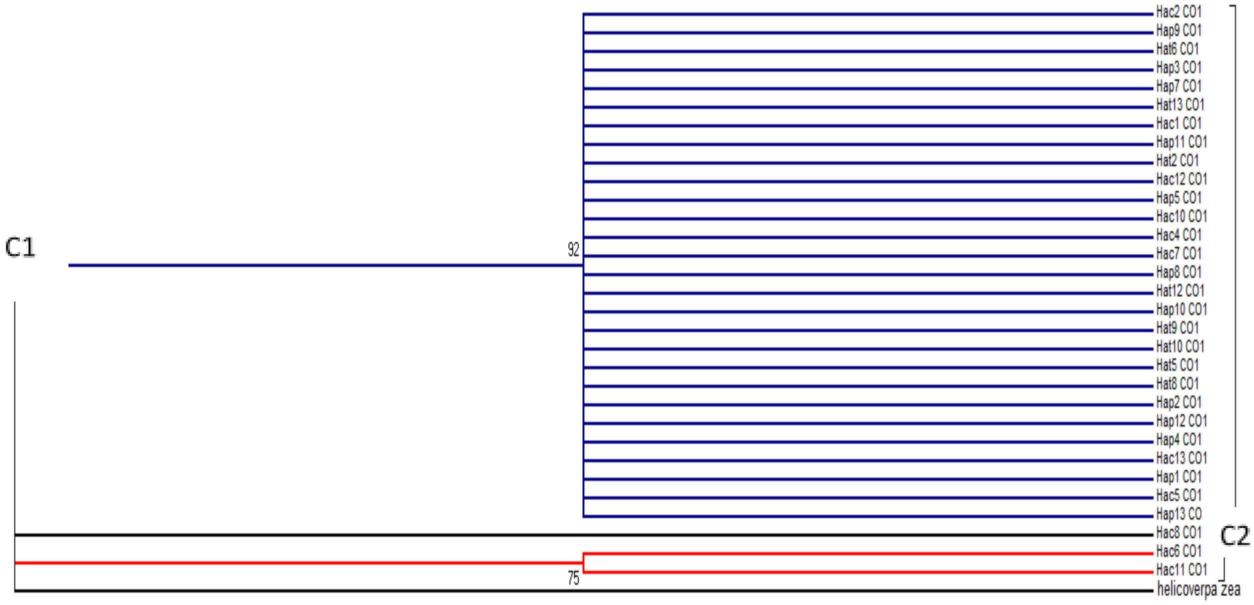


*Figure 3:* Phylogenetic trees of *H. armigera* individuals by the Neighbor-joining method

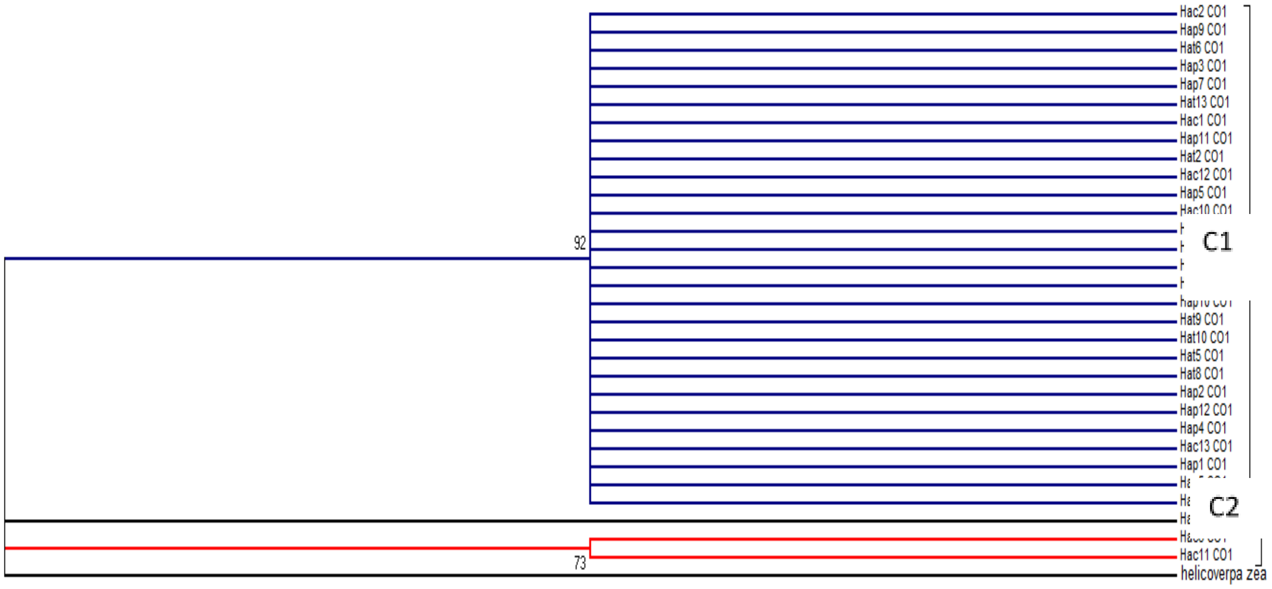


*Figure 4:* Phylogenetic trees of *H. armigera* individuals by the Maximum Parsimony method

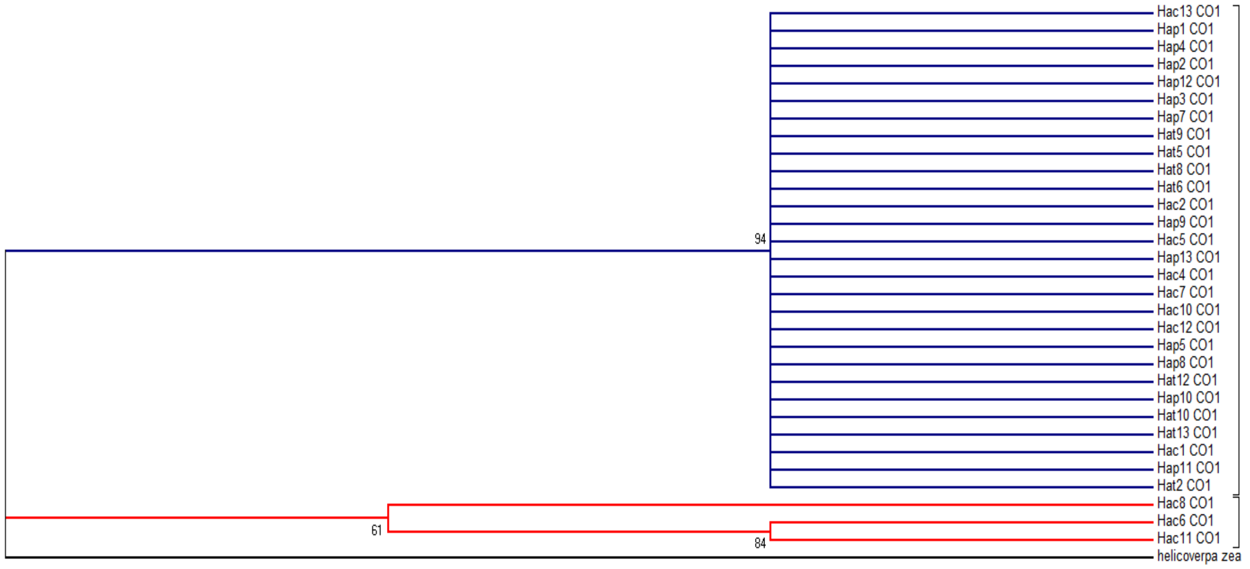


*Figure 5:* Phylogenetic trees of *H. armigera* individuals using the Maximum Likelihood method

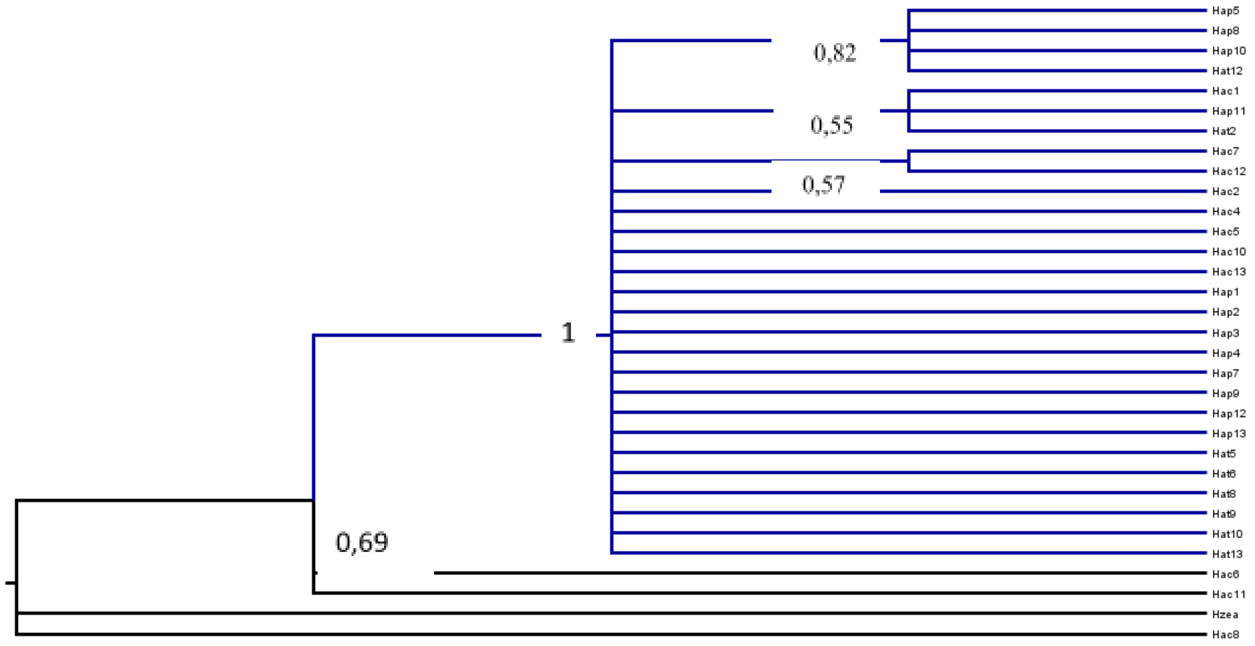


*Figure 6:* Phylogenetic tree of individuals of *H. armigera* by the Bayesian method

### 3.2. Discussion

The general objective of this study is to know the genetic diversity and structure of the populations of *Helicoverpa armigera*, a pest of vegetable crops in the Niayes area of Senegal. Analysis of the genetic diversity of *H. armigera* sequences shows that the percentage of transversion (50.96%) is higher than the percentage of transition (49.06%). This leads to a change in amino acids and proteins. The population of *H. armigera* as a whole has high haplotypic diversity and low nucleotide diversity. This could be explained by the signal of rapid population growth from a low-sized ancestral population that has been effective for a sufficient time for a restoration of haplotypic diversity (hd) by mutation, but too short for the accumulation of strong differences between sequences. Populations of *H. armigera* subservient to different plants (*B. oleracea*, *S. lycopersicum* and *C. annuum*) have high haplotypic diversity and low nucleotide diversity. This could be explained by a strong polyphagia, high fecundity [40] and rapid multiplication of the insect that is able to fill up to 10 to 11 generations per year [17]. This high haplotypic diversity could probably be linked to international trade [49] where imports of agricultural products (maize, sorghum ...) and horticultural (tomato, pepper, cabbage ...) result from the movement of goods. This commercialization allows the population of *H. armigera* from different localities to reproduce and form a population. This was found by [1] which stipulate that commercial movements may not serve as potential entry points for further incursions of agricultural pests. Analysis of the genetic differentiation (Fst) of *H. armigera* populations reveals that there is no genetic differentiation between the different populations of *H. armigera*. This could be explained by the strong migratory capacity of the insect *H. armigera* which can reach thousands of kilometers [16]. Indeed, the insect has a strong ability to spread through a connected landscape and via a hopping dispersion model [2]. These migrations can also allow exchanges between different host plants since the insect *H. armigera* colonizes vegetable and rainfed crops and is able to attack at least 217 species or genera of host plants [35] and caterpillars have a very different impact on different host plants [38]. The Niayes area is characterized by the alternation of two seasons: a wet season and a dry season [12]. Various crops are grown by farmers and *H. armigera* attacks a certain number of horticultural crops in the dry season but tomatoes are the main preferred host plant of *H. armigera*. During the rainy season, the main crops attacked by *H. armigera* are maize, cotton, and sometimes a few individuals attack groundnuts and cowpeas [37]. Given that in the Niayes area, most horticultural crops are practiced from September to June and *H. armigera* colonizes perimeters of vegetable crops such as tomato, cabbage, pepper, lettuce, eggplant ... and peaked in May. Rainfed crops are recolonized by *H. armigera* at the beginning of each rainy season. This asynchronous colonization has been described in West Africa by [37]. Analysis of molecular variance (AMOVA) of the COI gene of *H. armigera* individuals indicates a lack of structuring between host plants. This lack of genetic structuring has been confirmed by [36] which indicates an absence of genetic structuring on a geographical scale. This could lie in a recent extension of the insect's range: there would therefore be admixture between the populations of the different host plants. Indices of demographic change in *H. armigera* populations reveal p-value values well above 5%, which are therefore not significant. This makes it possible to say that the population is in demographic equilibrium or in moderate expansion. Analysis of the distribution of genetic distances between individuals in a population taken two by two (Mismatch distribution) reveals a multimodal curve that indicates a stable population. So the detected expansion would be moderate. The sum of the squares of the deviations (SSD) and the irregularity index (Rag) of the populations of *H. armigera* confirm the hypothesis of a demographic expansion with insignificant p-value values which makes it possible to say that there is a gap between the observed values and those simulated hence the acceptance of the zero hypothesis Ho which indicates that the population is in demographic expansion. Overall, the four methods of phylogenetic reconstructions (Neighbor-joining, Maximum Parsimony, Maximum Likelihood and Bayesian Inference) show congruent results. Indeed, they reveal that individuals are not structured according to the host plant.

## IV. CONCLUSION AND PERSPECTIVES

The importance of this study is linked to the fact that it provides the first insights into the genetic diversity of *Helicoverpa armigera* in the Niayes area of Senegal. A great haplotypic diversity was observed between the different host plants. A lack of genetic differentiation was also noted between the populations of the different host plants. Similarly, a lack of genetic structuring between the populations of the different host plants (*B. oleracea, S. lycopersicum* and *C. annuum*) was noted by a small percentage of variation between the host plants. Demogenetic tests indicate the hypothesis of an expanding population and this hypothesis is confirmed by the sum of the squares of the deviations (SSD) and the irregularity index (Rag) of the populations of *H. armigera*. The diversity between individuals from the same host plant deserves to be deepened. Thus, for a better understanding of the genetic structuring of *H. armigera* in Senegal it is necessary to:

- Expand sample sizes in different host plants to gain insight into the genetic diversity of this insect;
- Characterize the genetic structuring of *H. armigera* using other markers such as microsatellites to determine existing gene flows between host plants in Senegal.

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