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Dynamic Response, X-Raying the Genetic Structure and Population of T*uta Absoluta:* Using North Western Nigeria as a Case Study

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Abstract: *Ten Microsatellite loci for Tuta absoluta were developed for twenty- eight samples collected from North-Western part of Nigeria, in the states of Kano, Kastina, Kaduna, Sokoto, Kebbi, Jigawa and Zamfara which were helpful in the study of genetic structure of the affected tomato seeds.The Genomic Deoxyribonucleic Acid (DNA) of tomato seed was isolated using cetyltrimethylammonium bromide(CTAB) method.Tomato seeds were grinded in 600 μ L of extraction buffer and it was incubated at 65^0 C for 20 minutes. Agarose gel and electrophoresis were used to determine the quality and integrity of the DNA by size fractionation on 1.0% agarose gels. The result of the study shows that, two of the ten polymorphic micro satellites TG-22 and GTT-6 are generally informative to study genetic structure and equally to estimate the population of the pest in Mairuwa Faskari and Jibia Local Governments of Katsina State alongside Mada and Bungudu Local Governments of Zamfara State. However, the menace were found to be absent in other states under study.*

Keywords: *Genetic structure; microsatellite; Tuta absoluta.*

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I. Introduction

Tomato is considered one of the vital vegetable crops of the world and also one of the most researched of all horticultural crops and so far a considerable and progressive success has been made in this aspect(Ajibade *et' al.,* 2017). This is so as a result of its nutritional values which could either be consumed fresh or raw material for food processing industries (Gebremariam, 2015). Tomato is the world's largest vegetable crop after sweet potato but it tops the list of canned vegetables (Olaniyi, *et' al., 2010*). It is an important ingredient in most diets and a very cheap and very good source of vitamins A, C and E and minerals that are very good for body and protect the body against diseases (Taylor, 1987). It also contains a large quantity of water (75 %), calcium (20 %) and Niacin (Ajibade *et' al.,* 2017) all of which are of great importance in the metabolic activities of man (Ajibade *et' al.,* 2017). Lycopene is a carotenoid that is present in tomatoes, processed tomato products and other fruits. It is one of the most potent antioxidants among dietary carotenoids. Dietary intake of tomatoes and tomato products containing lycopene has been shown to be associated with a decreased risk of chronic diseases, such as cancer and cardiovascular disease (Agarwal and Rao, 2000).

The tomato leaf miner also known as South American Tomato pinworm, *Tuta Absoluta*Medeiros (2007) (Lepidoptera Gelechiidae) hails from South America, where it was first recorded as serious pest of tomato. It was experimented that a female *Tuta Absoluta* lays up to 260 eggs individually on the tender leaves during its life time (Desneux*et' al.,* 2010). *Tuta Absoluta* according to Medeiros (2009) was then later accidentally introduced to space in 2006 where it spread North to the Netherlands and East to Iran (Desneux, *et' al.,* 2010, 2011). It travels and breeds in swarms and has a reputation for swiftly ravaging tomato cultivation in a little above 48 hours prompting famers to nickname it tomato ebola. The moth and its larva feed on the leaves of the tomato plant depriving it of the nutrient to flower and to develop fruit (Larraín*et al.,* 2014).

In recent years, microsatellites markers have proven to be very powerful in studying genetic structure of many insect populations such as *Myzuspersicae*(MingHua*et al., 2009*);*Aphisspiraecola*(Cao *et al., 2012)* and *Bactroceradorsalis*(Isasawin*et al., 2012).* In our recent publication, we gave an overview of the origin of this devastating pest across different region in the world (Ajibade *et al.,*2017).

In the current study, we have developed microsatellite loci for *T. absoluta* for samples collected from north Western part of Nigeria which may be helpful in the study of its genetic structure and the population during invasion.Microsatellite loci is a short strand of DNA that serves as a starting point for DNA synthesis. It

is required for DNA replication because the enzymes that catalyses this process,DNA polymerases, and reveals its DNA sequence identity

II. Materials And Methodology

2.1 Genomic Deoxyribonucleic Acid (DNA)Extraction

The genomic DNA of tomato seed was isolated usingcetyltrimethylammonium bromide CTAB method (Doyle and Doyle 1987). Tomato seeds were grinded in 600 μL of extraction buffer and it was incubated at 65^0C for 20mins. The sample was removed from the incubator and allowed to cool to room temperature and Chloroform was added, the sample was mixed by gently inversion of the tube several times. Thereafter, the sample was spun at 14,000rpm for 15mins and the supernatant was transferred into a new eppendorf tube and equal volume of cold Isopropanol was added to precipitate the DNA. The sample was kept in the freezer for 1hr and later spun at 14,000rpm for 10mins and the supernatant was discarded and the pellet was washed with 70% ethanol later the sample was air dried for 30mins on the bench. The pellet was re suspended in 100 $\mu L$of sterile distilled water.

DNA concentration of all the samples was measured on Nanodrop and the genomic purity was determined. The genomic purity was between 1.8 –2.0 for all the DNA samples. The quality of DNA was detected by agarose gel electrophoresis and the size of fragment obtained was about 25kb for all the samples. The genomic DNA was used in PCR amplification using SSR markers.

2.2 DNA Electrophoresis

Agarose gel electrophoresis was used to determine the quality and integrity of the DNA by size fractionation on 1.0% agarose gels. Agarose gels were prepared by dissolving and boiling 1.0g agarose in 100ml 0.5X TBE buffer solution. The gels were allowed to cool down to about 45^0C and 10 μL of 5mg/ml ethidium bromide was added, mixed together before pouring it into an electrophoresis chamber set with the combs inserted. After the gel has solidified, 3 $\mu L$of the DNA with 5 μL sterile distilled water and 2 $\mu L$of 6X loading dye was mixed together and loaded in the well created. Electrophoresis was done at 80V for 2 hours. The integrity of the DNA was visualized and photographed on UV light source.

2.3 Dilution of DNA for PCR

About 100 μL of each DNA was taken into eppendorf tube and 900 μLsterile distilled water was added to make 1000 μL. The final concentration became 20-50ng/ μL.

2.4 PCR Reaction Mix

The reaction mix was carried out in 20 μLfinal volume containing 60ng– 80ng genomic DNA, 0.1 µM of the primers, 2mM $MgCl_2$, 125µM of each dNTP and 1 unit of Taq DNA polymerase. Thermocycler profiles used has initial denaturation temperature of 94^0C for 3mins, followed by 45 cycles of denaturation temperature of 94^0C for 20 seconds, annealing temperature of 37^0C for 40 seconds and primer extension temperature of 72^0C for 40 seconds, followed by final extension temperature at 72^0C for 5 min was added.

Twenty eight individual samples of affected seeds of *T. absolutawere* collected from different sites located in the North Western part of Nigeria in the states of Kano, Kastina,Kaduna, Sokoto, Kebbi, Jigawa and Zamfara.

Table; 2 Selected individual samples for polymorphic loci

S/N	LOCAL GOVERNMENT AREA/STATE	S/N	LOCAL GOVERNMENT AREA/STATE
1	Dutse, Jigawa State	15	Mada, Zamfara State 01
2	Giwa, Kaduna State	16	Bugundu Zamfara State 02
3	Wamako, Sokoto State	17	Bugundu Zamfara State 03
4	Bakura, Zamfara State	18	Bakura Zamfara State 04
5	MairuwaFaskari, KatsinaState	19	BakuraZamfara State 02
6	Jega, Kebbi State	20	Mada Zamfara State 02
7	ShagariSokoto State	21	MafaraZamfara State 01
8	Alero,Kebbi State	22	JibiaKatsina State01
9	JibiaKatsina State	23	Bakura Zamfara State 03
10	Gwarzo, Kano State	24	MafaraZamfara State 03
11	Bugundu, Zamfara State 04	25	Mada Zamfara State 03
12	MadaZamfara State 04	26	JibiaKatsina State 04
13	JibiaKatsina State02	27	BakuraZamfara State 01
14	Mafara Zamfara State 02	28	JibiaKatsina State03

PCR amplicon electrophoresis was carried out by size fractionation on 1.2% agarose gels. Agarose gels were prepared by dissolving and boiling 2.4g agarose in 200ml 0.5X TBE buffer solution. The gels were allowed to cool down to about 50^0C and 10 μ *L*of 5mg/ml Ethidium bromide was added, mixed together before pouring it into an electrophoresis chamber set with the combs inserted. After the gel has solidified, the PCR amplicon was loaded in the well created. Electrophoresis was done at 100V for 2 hours. The DNA was visualized and photographed on UV light source.

III. Results And Discussion

A total of 28 clones were found to contain microsatellitemotifs. After discarding sequences with few repeat regions and those not suitable for designing primers, 10 sequences were selected. Finally, 10 polymorphic loci were isolated and characteristics of each locus are shown in table 1. Levels of polymorphism were tested on 28 individual samples of *T. absoluta*collected on tomato from several regions in North Western Nigeria.

Table: 1 List of the primers used

S/N	NAMES	SEQUENCES
1	LC01490	
	GGTCAACAAATCATAAAGATATTGG	
	HC02198	
	TAAACTTCAGGGTGACCAAAAAATCA	
2	COI-F	TTGATTTTTTGGTCATCCAGAAGT
	COI-R	
	TCCAATGCACTAATCTGCCATATTA	
3	TG-14F	CGAAAAGTGAAAGCAAGGAC
	TG-14R	
	TCCTCATTTAACATTCTGAGGTTTT	
4	AC-16F	TCGGGAAAATGAAAATGTCAC
	AC-16R	GCCTAGCTAGCAATCACG
5	GTT-6F	GAATCCCAAGTTACCGCCC
	GTT-6R	ATTCCCGTAAACTTGGAAACA
6	TACA-6F	TCGCAAGCTTTTCAAATCAA
	TACA-6R	TCTGACACCAGGGTTCATCC
7	TG-22F	CGTAAGGGAAATAGGCGTGA
	TG-22R	AGCAGGCGTAGACCTTGGTA
8	CAA-7F	CGACAACGGAAGTGTCATGT
	CAA-7R	ACGTCAAATCGCATGGAAA
9	GTAT-5F	CTTACCATCCACCACCAAGG
	GTAT-5R	ATTTGCGCAATCCCAGATAG
10	GTTT-4F	
	AACTTAACCATTCAACTGATCAACA	
	GTTT-4R	GGTCTAGTAGTTTGGAGCCTGT

The result reveals that ,two of the ten polymorphic microsatellites namely TG-22 and GTT-6 for Primer TG-22are generally informative to study genetic structure and to estimate gene flow of the invasive tomato leaf miner.*Tuta absoluta* populations in Mairuwa Faskari and Jibia Local Governments of Katsina State alongside Mada and Bungudu Local Government in Zamfara State were revealed.Katsina takes the lead followed by Zamfara State

The corresponding code with the bandwidth, are 13, 15, 16 and 26 with 200bp .Sample 5 has two unique bands at 400bp and 450b in plate 1of figure 1 that shows the population density of the leaf miner.*Tuta absoluta.*

M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 2223 24 25 26 27 28 M

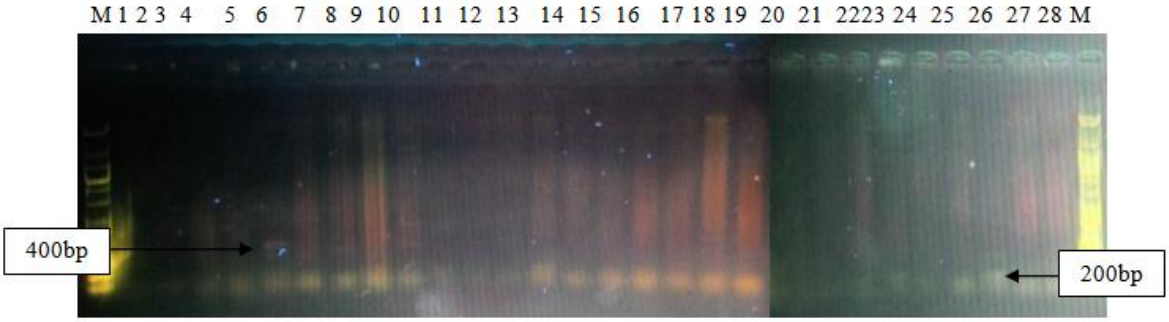

Figure 1: Plate1; Electrophoresis gel for TG-22

For Primer GTT-6, there are four samples with bandwidth 400bp, the samples are 3, 12, 13 and 26. Sample 5 has two unique bands at 700bp and 750bp in Plate2 of figure 2. This equally corroborates the genetic structure and the population density of the presence of the pest.

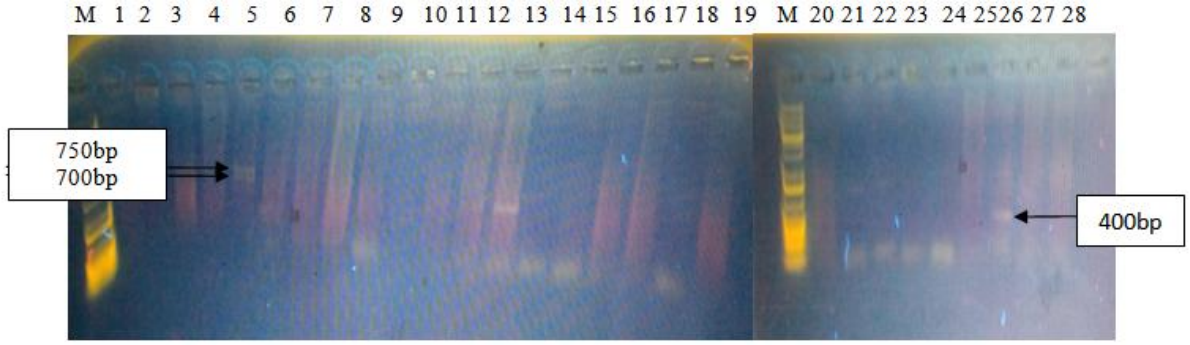

Figure 2: Plate2; Electrophoresis gel for GTT-6

IV. Conclusion

In this work,we have been able to show vividly from our research that out of twenty eight samples of the affected seed selected from seven Norther states in Nigeria,Four were confirmed having the presence of the devastating pest. The Genomic Deoxyribonucleic Acid **(**DNA) of the affected tomato seed was isolated using *cetyltrimethylammonium bromide*(CTAB) method.The seeds were grinded in 600 μ L of extraction buffer and it was incubated at 65^0 C for 20 minutes. Agarose gel electrophoresis was used to determine the quality and integrity of the DNA by size fractionation on 1.0% agarose gels. The result of the study shows that, two of the ten polymorphic micro satellites TG-22 and GTT-6 are generally informative to study genetic structure and equally to estimate the population of the pest in Mairuwa Faskari and Jibia Local Governments of Katsina State alongside Mada and Bungudu Local Governments of Zamfara State. However, the menace were found to be absent in other states under study.

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