

GENETIC VARIABILITY OF SPIRALLING WHITEFLY *ALEURODICUS DISPERSUS* RUSSELL ON *CITRUS AURANTIFOLIA* CHRISTM AND *OCIMUM GRATISSIMUM* L.

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ABSTRACT

The spiralling whitefly, *Aleurodicus dispersus* Russell (Hemiptera: Aleyrodidae), has a wide range of anatomy, physiology, behaviour, and ecology depending on the host plant. This research looked at the genetic variations between *A. dispersus* populations on two separate host plants (*Citrus aurantifolia* and *Ocimum gratissimum*). The existence of host-related genetic variation in *A. dispersus* populations was determined using Rapid Amplification of Polymorphic DNA (RAPD) markers. Our findings revealed that the *A. dispersus* populations on the two host plants had a considerable amount of genetic divergence. The spiralling whiteflies on the adaxial part of *Citrus aurantifolia* were genetically distinct from those on the abaxial part of the same plants. Various population genetic parameters such as heterozygosity, Nei's genetic gap, and fixation indices (*F_{ST}*) revealed that spiralling whitefly populations vary genetically, which may be attributable to spiralling whitefly populations originating from multiple sources. These findings also have consequences for the invasive pest's quarantine safety strategy.

Key words: *Aleurodicus dispersus*, Hemiptera, Aleyrodidae, genetic diversity, host plants, RAPD markers, polymorphism

INTRODUCTION

Citrus is one of the world's most essential fruit crops, with widespread abundance and popularity, adding to human diets as a rich source of fibre, carbohydrates, vitamins, and minerals that are essentially important for the maintenance of human health and growth. Citrus fruits are eaten fresh or refined into citrus by-products such as flavourings all over the world (Yano *et al.* 1999); jams, sorbets, pickles, jellies, and candies (Okwu, 2008) and Citrus fruit peel oils are used to flavour beverages and

sweets, as well as medications, soaps, perfumes, hair cream, body oil, and other cosmetics, as well as cleaning products. (Ferguson, 1990). Citrus fruits are cultivated in more than 140 countries around the world, mainly in the tropics and subtropics. Oranges, lemons, limes, grapefruit, and tangerines are the most well-known commercial citrus fruits.

C. aurantifolia is a native of southwest Asia and is known as Lime (Nigeria), Key lime, Mexican lime, Sour lime, Dayap, bilolo, Indian lime, and Egyptian lime (Burkill, 1997). The plant belongs to the

Phylum: Magnoliophyta; Class: Magnoliopsida; Order: Sapindales; Family: Rutaceae; Genus: Citrus and Species: Citrus aurantifolia (Sethpakdee, 1992). On an estimated 3 million hectares of land in Nigeria, around 930,000 tons of citrus fruits are produced annually (FAO, 2007), with production coming from Benue, Nassarawa, Kogi, Ogun, Oyo, Ebonyi, Kaduna, Taraba, Ekiti, Imo, Kwara, Edo, and Delta. (Taiwo, 2005).

Lime is a spiny shrubby or small tree with a single or several stems and irregular branches covered in smooth brown to grey bark that grows up to 6 meters tall (Aprioku and Obianime, 2014). The fruits are 3 to 5cm in diameter and have a juicy, greenish-yellow flesh that turns yellow when they mature. They also have a few white, pointed seeds that are around 1cm long (Katz and Weaver, 2003).

The pericarp (rind) of *C. aurantifolia* contains 7% essential oil, with key constituents such as citral, limonene, and fenchon, as well as terpineol, bisabolene, and other terpenoids including terpineol, bisabolene, and other terpenoids (Bruneton, 1999). Its main applications include food seasoning, refreshing beverages, delectable desserts, and seasoning foods, fruits, salads, sauces, and casseroles (Katzner, 2002). Lime is used to treat a huge number of ailments (Burkill, 1997), such as colon cancer, cardiovascular diseases, obesity, kidney stones, gout, arthritis, to relieve flu by taking tea prepared from juice, fruit rind or leaves as an expectorant (Rafter, 2002). The juice and peels of fruits are mixed with vinegar to create a disinfectant; polish brass; bleach cloth; remove ink, rusty, and mineral stains from cloths; whiten tannin shoes; and

soften fabrics (Ferguson, 1990). Citrus fruit peel pulp is used as a cereal substitute in ruminant feeds because of its high energy content and ruminant digestibility (Heuzé *et al.*, 2012).

Vulnerability of *C. aurantifolia* to pests and diseases is high just like other citrus trees, pests include; citrus snow scale (*Unaspis citri*), spiralling whitefly (Manner *et al.* 2006), citrus leafminer (*Phyllocnistis citrella*), citricola scale (*Coccus psuedomagnoliarum*), citrus leafroller (*Archips argyrospila*) and aphids (Weinblatt, 2007).

Ocimum gratissimum (African basil or Basil fever plant or tea bush) is an herbaceous plant food (vegetables) in developing countries, especially Nigeria that makes a significant contribution to local diets and ethnomedicine (Okigbo and Ogbonna, 2006). It is an aromatic, perennial plant native to Africa, Madagascar and southern Asia (USDA, 2013). *O. gratissimum* contains an essential oil, alkaloids, glycosides, tannins, and other chemical constituents (Pandey *et al.* 2014). Food flavoring, biological activity such as antidiabetic antiseptics, antitussive, anthelmintic, antispasmodic, and antimicrobial are just a few of the health benefits (Gbolade, 2009). It has also been proved to induce abortion (Nikolajsen *et al.* 2011), facilitate childbirth (Attah *et al.* 2012), manage diabetes (Ejike *et al.* 2013) and alleviate headache as well as fever (Nweze, 2009).

Aleurodicus dispersus Russell (Spiralling Whitefly) is a major pest devouring agricultural crops as it spread with extraordinary speed across tropics (plate 1). Spiralling whitefly develops as colonies of individuals on the underside of the

leaves where by the nymphs and adult suck the sap from the leaves. The polyphagy of the white spiralling whitefly is the major reason it is able to spread quickly from one crop to another across the tropics (Inbar and Gerling, 2008). Furthermore, this whitefly is a threat to many plants which

includes; banana (*Musa sapientum*) *Citrus spp*, coconut (*Cocos nocifera*) guava (*Psidium quajava*), tomato (*Lycopersicon esculentum*), pawpaw (*Carica papaya*), and African basil (*Ocimum gratissimum*) (Okolle *et al.* 2010; Sundararaj and Selvaraj, 2017; Kapantaidaki *et al.* 2019).



Plate 1: Sooty mould of *A. dispersus* infestation on *O. gratissimum* (A) and *C. aurantifolia* (B)

Direct feeding damage is also caused by immature and adult stages of whiteflies piercing and sucking sap from foliage (Boopathi *et al.* 2013). When feeding, dense populations of this polyphagous pest cause premature leaf drop and abundant honeydew, which serves as a substrate for the growth of sooty fungus, causing cassava fields to be abandoned (Akinlosotu *et al.* 1993). Understanding the invasion process requires knowledge of the genetic makeup of invading insect populations (Kreiser *et al.* 2000; Cristescu *et al.* 2001; Patti and Gambi, 2001), since significant genetic variability is expected to favour adaptation and, as a result, the successful establishment of introduced populations (Lee, 2002; Kolbe *et al.* 2004; Facon *et al.* 2006; Lavergne and Molofsky, 2007).

Genetic variability is a measure of individual genotypes in a population in order to determine if the population differ

from one another (Sousa *et al.* 2011). This is distinct from genetic diversity, which refers to the amount of variation found in a population (Ehrich and Per Erik, 2005). Variability is an important factor in evolution because it influences an individual's reaction to stress in the environment, resulting in differential survival and natural selection of the fittest variants. (Linhart and Janet, 2003).

Genetic markers such as RAPD, Amplified Fragment Length Polymorphism (AFLP), have been an efficient tool to differentiate geographically and genetically population (Subodh *et al.* 2010). Analysis of microsatellite and allozyme markers on plant pest indicates the existence of genetically different individuals on the various plants (Simon *et al.* 2003). It's been used to test the genetic diversity of insect pests like the sweet potato whitefly (*Bemisia tabaci*) in Indian (Moya *et al.*

2001; Sharma *et al.* 2008), grain aphids *Sitobion avenae* (Lushai *et al.* 2002), Russian wheat aphid *Diuraphis noxia* (Shufran *et al.* 1997), whitefly *Aleurocanthus spiniferus*) in Greece (Kapantaidaki *et al.* 2019) and giant whitefly *Aleurodicus dugesii* in Indonesia (Hidayat *et al.* 2020) However, considering its economic value, little is known about *A. dispersus*' genetic variability level and pattern. Moreover, the level of genetic divergence among host plant specific *A. dispersus* populations is uncertain because in earlier studies the populations were analysed using markers with different resolutions. As a result, we examined the extent of genetic differentiation between *A. dispersus* populations on two host plants in this study.

METHODS

Two hundred (200) insect samples (active spiralling whiteflies) were collected from various parts of leaves of *Citrus aurantifolia* (adaxial and abaxial surface) and *Ocimum gratissimum* (abaxial surface) early in the morning (7am) with improvised pooter into sterile bottles. The plants and insects species were identified at the herbarium and Entomology laboratory

in Plant and Applied Zoology of Olabisi Onabanjo University, Ago-Iwoye, Ogun State. The samples were then preserved in the refrigerator for 24 hours for further analysis at the Animal Science Laboratory of Federal University of Agriculture, Abeokuta, Ogun state.

The extraction of the genomic DNA of *A. dispersus* was done using CTAB (Cetyl trimethyl ammonium bromide antiseptic agent) extraction buffer method (Cortes *et al.* 2010). Purity and quantity of extracted DNA were tested using a spectrophotometer, and purity was checked using a 1% agarose gel electrophoresis, which was visualized using an ultraviolet light transmitter and stored at -20°C.

DNA AMPLIFICATION: Five (5) RAPD markers namely OPAD 09, OPAE 04, OPAE 05, OPAE 09 and OPAF 07 (Table 1) were used to amplify the DNA samples that had been collected. PCR was performed according to Solignac *et al.* (2003). Electrophoresis in a 6.0 % polyacrylamide gel with 1.0 X TBE buffer was used to overcome the amplification materials. Gels were stained with ethidium bromide solution after electrophoresis.

Table 1: List of primers used and their sequences

S/N	Primer Name	Sequence	Fragment size
1	OPAD 09	TCGCTTCTCC	200bp - 2500bp
2	OPAE 04	CCAGCACTTC	200bp - 2500bp
3	OPAE 05	CCTGTCAGTG	250bp - 2500bp
4	OPAE 09	TGCCACGAGG	250bp - 3000bp
5	OPAF 07	GGAAAGCGTC	250bp - 3500bp

DATA ANALYSIS

For RAPD studies, polymorphic bands were scored as present (1) or absent (0). Shannon's knowledge indices (I), observed heterozygosity (H_o), gene diversity (H_e) (Nei, 1973), and percent of polymorphic bands (Lewontin, 1972) were calculated using the following equation:

$$I = -\sum p_i \ln p_i$$

where p_i denotes the population proportion of the i th allele.

The binary value was transferred into software for constructing the UPGMA tree based on the original distance PAST v.4.0.3 (Miller, 1997)

RESULTS

All amplification products were consistent and reproducible in the RAPD analysis. This revealed genetic diversity among the spiralling whitefly populations collected from the two (2) host plants. In the RAPD amplifications, a total of twenty alleles were detected from the two host plants however, only ten were polymorphic and were used in the analysis (Table 2).

Table 2: Observed number of bands, percentage of polymorphic bands, observed heterozygosity (H_o), Gene diversity (H_e) and Shannon's information index (I) values for populations studied.

LOCUS	Observed	% Polymorphic	H_o	Gene diversity (H_e)	I
OPAD 09	10	50	0.02	0.34	1.089
OPAE 04	12	60	0.02	0.36	1.099
OPAE 05	3	15	0.01	0.19	0.637
OPAE 09	7	35	0.02	0.3	1.079
OPAF 07	9	45	0.02	0.33	0.965
Mean			0.018	0.304	0.762

Per locus, the number of alleles ranged from 0 to 4, with 2 alleles per locus on average. The percentage polymorphism ranged between 15% (OPAE 05) and 60% (OPAE 04). The observed heterozygosity (H_o), Gene diversity (H_e) and Shannon's information index (I) ranged from 0.01 to 0.02 (mean = 0.018), 0.19 to 0.36 (mean = 0.304) and 0.64 to 1.10 (mean = 0.76), respectively. It is noteworthy that the observed heterozygosity is much lower than the expected heterozygosity.

The most informative primers were OPAD 09 and OPAE 04, they have the highest number of expressed bands (10 and 12 respectively) in 20 loci. The organisms with highest number of polymorphic expressions were those obtained from *O. gratissimum* (16 bands) and *C. aurantifolia* (adaxial) (13 bands).

The neighbour joining dendrogram of *A. dispersus* populations from the two host plants based on Nei's genetic distances revealed two major clusters (Figure 1). The mean genetic distance coefficient based on

four markers is 0.99. Spiralling whitefly populations on the *C. aurantifolia* (adaxial) is of same origin with those found in *O.*

gratissimum host plants forming a major cluster. The second cluster consists of *C. aurantifolia* (abaxial).

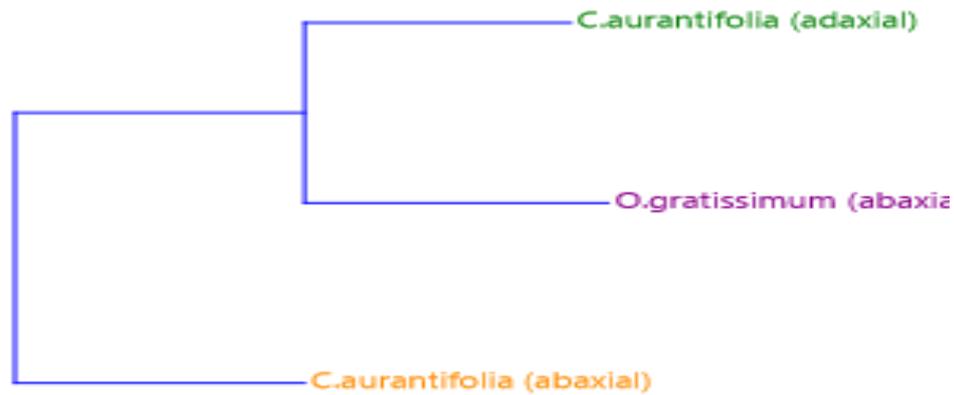


Figure 1: Neighbour joining dendrogram for populations of *Aleurodicus dispersus* collected from two host plants based on Nei's (1973) genetic distances generated using PAST 4.03.

Pairwise F_{ST} , which estimates the correlation of alleles among hypothetical populations of spiralling whiteflies, indicated that population *C. aurantifolia* (adaxial) is genetically closer to *O. gratissimum* (abaxial) than the other population (Table 3).

Table 3: Pairwise F_{ST} correlation matrix for spiralling whitefly populations based on the clusters identified by the PAST analysis.

Host Plant	<i>C.aurantifolia</i> (adaxial)	<i>C.aurantifolia</i> (abaxial)	<i>O. gratissimum</i> (abaxial)
<i>C.aurantifolia</i> (adaxial)	1		
<i>C.aurantifolia</i> (abaxial)	0.778	1	
<i>O. gratissimum</i> (abaxial)	0.9649	0.6447	1

DISCUSSION

The first step toward a deeper understanding of insect ecology at the molecular level is to define, validate, and apply molecular markers. This study has revealed spiralling whitefly colonising abaxial surface of leaves of *O. gratissimum* and both the abaxial surface and adaxial

surface of leaves of *C. aurantifolia* are widely infested in acceptance with Chin *et al.* (2006) that reported spiralling whitefly colonization of both the underside and upper (adaxial) surface of leaves. Despite the fact that there is no morphological variation between the insect populations on the adaxial and abaxial surfaces of the

leaves, the size of the insect population on *O. gratissimum* is smaller.

The RAPD analysis clearly demonstrated a substantial level of polymorphism among the populations of spiralling whiteflies. Twenty multiple alleles were generated from *C. aurantifolia* (adaxial surface and abaxial surface) five alleles being polymorphic and ten alleles expressing polymorphism between *C. aurantifolia* and *O. gratissimum*, this indicates clear differentiation between the samples collected from *C. aurantifolia* (abaxial and adaxial) and *O. gratissimum* (abaxial) surface of leaves. Following the analysis of this study, it was revealed that the similarity coefficient of *A. dispersus* colonising the adaxial and abxial leaf surface of *C. aurantifolia* is 78% similar in their genetic makeup and 96% between *C. aurantifolia* and *O. gratissimum*

. This is in line with an analysis of host-associated genetic variation in *Bemisia tabaci* in India, which found a high degree of genetic relatedness (72% to 85%) among the whitefly types studied and the lineage of their origin from the common type (Sharma *et al.* 2008; Boopathi *et al.* 2019). Furthermore, in an analysis of genetic trends in *A. dispersus* populations from the Canary Islands, no variations in RAPD patterns were found, but there was a high degree of genetic similarity and low genetic variability among populations (Callejas *et al.* 2005). Oyelade and Ayansola (2015) have justified that this outcome can be aided by the favourable conditions and nutrient availability for the pest.

The use of ten markers in the genetic variability analysis of whitefly populations revealed substantial variability among

whitefly populations, with an observed mean heterozygosity (H_o) of 0.018 and average expected heterozygosity (H_e) of 0.304. In contrast, Ma *et al.* (2011) reported using microsatellite markers, a higher level of heterozygosity in populations of spiralling whiteflies in the Hainan and Canary Islands, which may be due to differences in the environment, population behaviour or shorter distances between samples. Expected heterozygosity is a prediction based on the assumption that the population is in Hardy-Weinberg equilibrium (HWE). Lower H_o than H_e indicated that the populations of spiralling whiteflies are smaller in size or have undergone a severe bottleneck in recent times. Another reason for this may be that the populations often do not outcross, which may vary from species to species. Substantial divergence among the populations of *A. dispersus* might be due to parallel multiple introductions of already diverged populations of the species from different sources and further divergence due to preferences for different host species (Boopathi *et al.* 2019).

From the Neighbour Joining clustering pattern it was evident that the genetic distances between *A. dispersus* populations do not correlate with the taxonomic relations of their host plants. There was an exception; where *A. dispersus* populations from *C. aurantifolia* (adaxial) and *O. gratissimum* were in the same clusters while populations from *C. aurantifolia* (abaxial) formed another cluster on its own despite being the plant belonging to the same family. This could have been due to the coexistence of flies from two distinct populations on a single host species as genetic and mixture resulting from inter-crossing is not expected to occur.

A high degree of gene diversity and a low level of reported heterozygosity (expected heterozygosity) indicated that the populations were not in HWE, which may be due to a high incidence of inbreeding and low incidence of intercrossing between individuals of distinct populations. This could be attributed to the result of sexual preferences between host plant specific populations and host plant-whitefly interactions.

The genetic data presented here, as well as the biological data on success and feeding behaviour presented in studies (Banjo, 2010; Oyelade and Ayansola, 2015; Boopathi *et al.* 2014) demonstrate that the genetical divergence of *A. dispersus* is associated with differences in the host plants.

Spiralling whitefly host specialization may have occurred before their introduction into any plant population, according to this research. Further research into the genetic relationships between and within *A. dispersus* host plants in various Nigerian geographical zones is required to decide if host specialization evolved once or many times.

CONCLUSION

The present study also utilized RAPD markers for identifying host correlated genetic variability amongst populations of *A. dispersus*. This research demonstrates unequivocally that *A. dispersus* on the two host plants differ substantially genetically and there is a minimum of intercrossing between these populations. In order to shed light on the host-race creation process in *A. dispersus*, it is essential to conduct phylogeographic study. More studies on molecular tools such as Restriction

Fragment Length Polymorphism, AFLP, Simple-Sequence Repeats, Single Nucleotide Polymorphisms as used by Boopathi *et al.* (2019) and Elfekih *et al.* (2018) respectively, and molecular sequences (mitochondrial and nuclear genomes) can be of great help to reinvestigate the genetic variability of *Aleurodicus dispersus* in Nigeria.

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