# Black Sigatoka Disease Indices and Responses of Hand & Open Pollinated Diploid & Tetraploid Banana & Plantain Genotypes

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Abstract- This study was conducted to investigate the black sigatoka disease indices (BSI) of hand and open pollinated 2x and 4x banana and plantain progenies under naturally occurring infection with a view to identifying resistant genotypes and to ascertain how BSI affects vegetative and yield components. Thirty six banana and plantain genotypes, comprising 6 2x and 6 4x parental clones, 6 2x hand pollinated and, 6 2x open pollinated progenies, 6 4x hand pollinated progenies and 6 4x open pollinated progenies were planted using a randomised complete block design with two replications of six plants per genotype. Data collected were total number of leaves produced, number of standing leaves at flowering, number of youngest leaf showing necrotic spots due to black sigatoka disease at flowering, plant height, plant girth, height of tallest following sucker; bunch weight, number of hands per bunch, number of fingers per bunch and yield. Leaf Retention Index (LRI) and Black Sigatoka Index (BSI) were estimated. Data were subjected to analysis of variance and means compared by LSD test at  $P \ge 0.05$ . There were significant differences (P > 0.05) in total number of leaves, number of standing leaves, number of youngest leaf showing necrotic spots, plant height, plant girth, height of tallest following sucker; bunch weight, number of hands per bunch, number of fingers per bunch and yield by some 2x and 4x parental clones and one or other of their progenies. In tetraploids, plant height was significantly associated with BSI ( $r = 0.502^*$ ), height of tallest following sucker ( $r = 0.534^*$ ), bunch weight ( $r = 0.551^*$ ) and yield  $(r = 0.477^*)$ . The diploids TMB2x 8084-2OP and TMB2x 8084-2HP showed potential of being good black sigatoka resistant genotype whereas high yielding tetraploids with good black sigatoka resistance were parental clones TMP4x 7002-1CL, TMP4x 1658-4CL and TMP4x 7152-2CL.

*Keywords:* - Musa spp, parental clones, necrosis, naturally occurring infection, vegetative traits, yield components

## I. INTRODUCTION

**B** lack Sigatoka disease also referred to as Black leaf streak disease is caused by the fungus *Pseudocercospora fijiensis* (formerly *Mycosphaerella fijiensis*) and is one of the most destructive diseases of plantain and banana accounting for yield losses of up to 30-50% and even up to 100% by the second ratoon crop [1], [2], [3]. Black Sigatoka starts as dark streaks, initially visible on the lower leaf surface. Streaks rapidly enlarge, forming the characteristic elongated black lesions, visible on both leaf surfaces [4]. These lesions become necrotic and quickly destroy large portions of mature leaf

tissue, causing severe defoliation and reduction in yield. The disease was first identified in Fiji Island [5], but it is now present in nearly all other tropical and sub-tropical production areas like Central and South America, North America, French West Indies, Asia, Australasia and Oceania and other islands in the Pacific region [6], [7], [8], [9], [10]. In Africa, black Sigatoka was first identified in Gabon in 1978, from where it spread westward to Cameroon in 1980 and Nigeria in 1986 [11], [12]. Since then infection risk has increased by more than 44% [13]. The socio-economic impact of this disease has continued to increase as the pathogen reaches new areas and the disease becomes more difficult to control [14]. Today contrary to some belief that the disease is no longer a threat, it is still migrating to new areas such as Reunion Island in Africa [15] and its spread worldwide is now being captured on maps [16], [17], [18]. Only a few countries like India, Pakistan, South Africa, Israel and mainland Australia that grow banana commercially for export are reportedly free of the disease [19]. In fact, [18] put it quite succinctly stating that the disease is a major constraint to global production of banana and plantain declaring that the bulk of global banana production experiences high potential threat from P. fijiensis, and the higher yielding areas for banana and plantain production are at greatest threat. Black sigatoka is more virulent, has a shorter life cycle and is harder to control than yellow sigatoka [20]. This is so because together with P. musae (previously M. musicola) causal agent of Sigatoka disease, and P. eumusae (previously *M. eumusae*) which is considered to be more aggressive than P. fijiensis [3] that causes Septoria leaf spot, they form the Sigatoka complex. These three pathogens can coexist on the same leaf or in the same lesion even along with other fungi of minor economic importance [21]. It is considered a biosecurity risk to the Australian banana industry [20], [22], [23], [24]. Infection is favoured by hot humid and windy weather [20], [22]. The disease reduces the photosynthetic efficiency of the plant, because the pathogen destroys the leaf area by the action of the phytotoxin, whose substance prevents the passage of electrons in the chloroplast membrane, causing foliar tissue necrosis [25]. There are six stages of symptom development [23] and symptoms can be quite similar to yellow sigatoka and to Eumusa streak. Australia has regained disease-free status for black sigatoka [22]. Despite the use of different control strategies, including good field practices such as early warning /forecasting systems, symptoms screening, biocontrol agents and leaf pruning, the only truly effective black sigatoka management relies on frequent fungicide applications [26]. To minimize yield loss, different fungicides e.g., dithiocarbamates, dithane M-22, benlate, strobilurins, morpholines and triazoles, petroleum oil, etc which must be applied from ground or by aircraft have been used to control the disease in large commercial farms [27], [28], [29], [30]. The fungicides and methods of application are expensive; accounting for up to 27% of production costs [31]; yet they do not provide long term control due to the destructive potential of black sigatoka. Moreover, they have negative effects on human health because they generate respiratory, endocrine, and skin problems, and the impact on the environment is high coupled with reported development of resistance by the pathogen to some fungicides after continuous use [27], [30]. Research has shown that silicon, applied to the soil as silicic acid or potassium silicate, helps reduce the progress of black sigatoka in banana [32, 33]. Also spraying of Lixiviate (a liquid produced by decomposing plantain harvest residues) on the leaves has proven useful in controlling the disease [34]. Other methods of control used to prevent the spread of the disease in small farms are destruction of infected plants and elimination of the lowest necrosed leaves /deleafing to reduce the spread of the disease [35]. Avoidance mechanism is also used in which organic bananas are grown in areas not conducive for P. fijiensis, such as in Peruvian highlands, and therefore do not require fungicide applications [36]. Generally, the cooking banana (ABB) cultivars exhibit varying reactions to the fungus, ranging from susceptibility to resistance [37]. In contrast, the plantains (AAB) and dessert bananas (AAA) show widespread susceptibility. Thus, genetic control, e.g., the use of resistant hybrids [38], [39], [40], [41] and use of GMO besides being the most economic and effective control measure, is also an environmentally safe and sustainable technology. However, the large-scale adoption of these hybrids is not always guaranteed due to differences in taste, consumer preferences, cost of adoption to farmers, and farmer predisposition in favour of some agronomic traits and processing qualities, amongst others [42], [43], [44]. One of the most efficient techniques to generate resistance to black sigatoka is the use of agrobacterium tumefaciens as a conventional method of genetic transformation. In particular, the use of recent innovative techniques such as CRISPR / Cas9 for gene modification would be a powerful tool to generate high resistance to diseases caused by fungi, maintaining banana quality and decreasing the high maintenance costs. Unfortunately, the application of techniques for genetic modification of plants is still restricted in some countries while others are yet to accept GMO's within their borders [30]. Low fertility, triploidy, slow propagation, space requirement and cycling time, among others have all contributed to a complex process that has not attracted manifold breeders and hence, the release of new attractive germplasm has been minimal over the last decade [45].

Therefore, banana and plantain improvement has largely concentrated on selection within clones and hybrids for disease resistance, for productivity, fruit flavor, hardiness, height, bunch weight and shelf life, etc. Evidently, under such conditions and circumstances, on top of the recent "orphan status" of bananas and plantains, progress on the genetic control of fungal diseases has been extremely slow and sporadic, and with regard to black Sigatoka, progress has been even more limited. This experiment was conducted therefore to investigate under naturally occurring infection, the black sigatoka disease indices (BSI) of hand and open pollinated diploid and tetraploid banana and plantain progenies with a view to identifying resistant genotypes in relation to their parental clones and how BSI affects vegetative and yield components.

## **II. MATERIALS AND METHODS**

This study was carried out at the International Institute of Tropical Agriculture (IITA) High Rainfall Station, Onne (4°51'N, 7° 03'E, 10m above sea level), in Rivers State, Nigeria. The rainfall pattern is monomodal, distributed over a 10month period from February through December, with an annual average of 2400mm. Relative humidity remains high all year round with mean values of 78% in February, increasing to 89% in the months of July and September. The mean annual minimum and maximum temperatures are  $25^{\circ}C$ and 27<sup>°</sup>C, respectively, while solar radiation / sunshine lasts an average of 4hours daily [46]. At fruit maturity (90 - 120)days following flower emergence), bunches from hand and open pollinated plants were harvested and ripened with ethylene for four days. Seeds were extracted from the ripened bunches and the embryos were excised and cultured in vitro for six weeks. The resulting diploid (2x) and tetraploid (4x)progeny seedlings were transferred to the nursery. Two-month old seedlings of these along with the parental clones (vegetatively propagated from parents) of each genotype (4x and 2x), were planted in the field. Thus a total of 6 diploid and 6 tetraploid parental clones, 6 diploid hand pollinated progenies, 6 diploid open pollinated progenies, 6 tetraploid hand pollinated progenies and 6 tetraploid open pollinated progenies were planted (Table 1). The experimental design was a randomised complete block design with two replications of six plants per genotype. Planting was done in alleys of multispecies hedgerows in an area of 2,880 m<sup>2</sup> at a spacing of 3m x 2m [47], [48]. Fertilizer was applied at the rate of 300kg N and 450kg K per hectare, split into six applications [49], [50]. Weeds were controlled with Paraquat (150 ml Gramoxone in 20L water), applied when necessary. Other cultural practices were the same as those previously described by [49] except that there was no effort made to control black sigatoka disease. Black Sigatoka disease progression was monitored under naturally occurring infection in the field.

| Diploids (2x)          |   |   | Tetraploids (4x)      |   |   |  |  |  |
|------------------------|---|---|-----------------------|---|---|--|--|--|
| Parental<br>Clones     | Hand<br>Pollinate<br>d<br>Progenie<br>s | Open<br>Pollinate<br>d<br>Progenie<br>s | Parental<br>Clones    | Hand<br>Pollinate<br>d<br>Progenie<br>s | Open<br>Pollin<br>ated<br>Proge<br>nies |  |  |  |
|                        | Plantains                               |   | Plantains             |   |   |  |  |  |
| TMP2x<br>1448-<br>1CL  | TMP2x<br>1448-<br>1HP                   | TMP2x<br>1448-<br>1OP                   | TMP4x<br>1658-<br>4CL | TMP4x<br>1658-<br>4HP                   | TMP4<br>x<br>1658-<br>4OP               |  |  |  |
| TMP2x<br>2625-<br>5CL  | TMP2x<br>2625-<br>5HP                   | TMP2x<br>2625-<br>5OP                   | TMP4x<br>2796-<br>5CL | TMP4x<br>2796-<br>5HP                   | TMP4<br>x<br>2796-<br>50P               |  |  |  |
| TMP2x<br>2829-<br>62CL | TMP2x<br>2829-<br>62HP                  | TMP2x<br>2829-<br>62OP                  | TMP4x<br>4698-<br>1CL | TMP4x<br>4698-<br>1HP                   | TMP4<br>x<br>4698-<br>1OP               |  |  |  |
| Bananas                |   |   | Plantains             |   |   |  |  |  |
| TMB2x<br>8084-<br>2CL  | TMB2x<br>8084-<br>2HP                   | TMB2x<br>8084-<br>2OP                   | TMP4x<br>6930-<br>1CL | TMP4x<br>6930-<br>1HP                   | TMP4<br>x<br>6930-<br>1OP               |  |  |  |
| TMB2x<br>8532-<br>1CL  | TMB2x<br>8532-<br>1HP                   | TMB2x<br>8532-<br>10P                   | TMP4x<br>7002-<br>1CL | TMP4x<br>7002-<br>1HP                   | TMP4<br>x<br>7002-<br>1OP               |  |  |  |
| TMB2x<br>9839-<br>3CL  | TMB2x<br>9839-<br>3HP                   | TMB2x<br>9839-<br>3OP                   | TMP4x<br>7152-<br>2CL | TMP4x<br>7152-<br>2HP                   | TMP4<br>x<br>7152-<br>2OP               |  |  |  |

Table 1. Diploid and Tetraploid bananas and plantains used in the experiment

CL = Parental clones; HP = Hand pollination; OP = Open pollination

Data Collection and Statistical Analyses under naturally occurring black sigatoka infection

Data on growth parameters, black sigatoka disease response and yield characteristics were recorded at flowering and harvest respectively for three crop cycles as follows:

- 1. Total number of leaves produced (TNL) leaves produced on the pseudostem at flowering, inclusive of dead and photosynthetically active leaves
- 2. Number of standing leaves at flowering (NSLF) total number of healthy leaves on the pseudostem at flowering
- 3. Number of Youngest leaf showing necrotic spots due to black sigatoka disease at flowering (YLSF) was also recorded. This was done, by numbering from the youngest fully expanded leaf downwards to the youngest leaf showing black spots, due to black sigatoka disease [4]
- 4. Leaf Retention Index (LRI) the number of standing leaves at flowering (NSLF) divided by the total number of leaves (TNL). The LRI is the proportion of photosynthetically active leaves at flowering
- 5. Black Sigatoka Index (BSI), was calculated as a measure of resistance to black sigatoka disease such that the higher the BSI, the higher the resistance to black sigatoka. The BSI indicates the proportion of

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standing leaves that are free from infection (BSI = YLSF/NSLF).

- 6. Plant height, plant girth, height of tallest following sucker and number of suckers;
- 7. Bunch Weight (kg), Number of hands per bunch, Number of fingers per bunch and Yield (tons/ha)

All data were subjected to the analysis of variance in a randomized complete block design using the General Linear Model (GLM) of Statistical Analysis Software (SAS, 2010) to test for significance at 5%, level of significance. If a measured trait was significant, the means were separated by the least significant difference (LSD) test at P = .05 and presented along with the 3 year means in tables. Crop cycle main effects were examined to determine how performances differed in the plant, first ratoon and second ratoon crops. Simple linear correlation analysis (r) was performed to find out the relationships between the Black Sigatoka indices and vegetative traits, yield and yield components.

#### **III. RESULTS AND DISCUSSION**

There were significant differences (P=.05) in the total number of leaves produced (TNL) by some 2x and 4x parental clones and one or other of their progenies (Table 2). For diploids such parental clones were TMP2x 1448-1CL, TMP2x 2625-5CL and TMB2x 8084-2CL and for tetraploids they include TMP4x 1658-4CL and TMP4x 4698-1CL. A similar but not identical pattern occurred in the number of standing leaves at flowering (NSLF). This is because whereas some parental clones differed significantly in the number of standing leaves they produced from one or other of their progenies, some hand pollinated progenies differed significantly from their open pollinated counterparts but not from their parental clones.

Table 2. Total Number of Leaves (TNL), Number of standing leaves (NSLF) and Number of Youngest leaves showing necrotic spots (YSLF) at flowering of Parental clones (CL) hand pollinated (HP) and open pollinated (OP) diploid (2x) and tetraploid (4x) Banana & Plantain genotypes under naturally

occurring infection by Black Sigatoka disease over 3 crop cycles at IITA High Rainfall Station, Onne, Rivers State, Nigeria

| Diploid<br>(2x)<br>Genotypes | Tetraploid<br>(4x)<br>Genotypes | Total<br>number of<br>leaves<br>(TNL) |          | Number of<br>standing<br>leaves at<br>flowering<br>(NSLF) |          | Number of<br>Youngest<br>leaves<br>showing<br>necrotic<br>spots at<br>flowering<br>(YLSF) |     |
|------------------------------|---------------------------------|---------------------------------------|----------|---|----------|---|-----|
|                              |                                 | 2x                                    | 4x       | 2x  | 4x       | 2x  | 4x  |
| TMP2x<br>1448-1 <b>CL</b>    | TMP4x 1658-<br>4 <b>CL</b>      | 38.0                                  | 37.<br>0 | 11.5  | 10       | 9.0   | 8.7 |
| TMP2x<br>1448-1 <b>HP</b>    | TMP4x 1658-<br>4 <b>HP</b>      | 33.0                                  | 35.<br>0 | 11.0  | 9.5      | 8.5   | 7.7 |
| TMP2x<br>1448-1 <b>OP</b>    | TMP4x 1658-<br>4 <b>OP</b>      | 27.5                                  | 29.<br>0 | 8.0   | 9.0      | 6.4   | 7.1 |
| TMP2x<br>2625-5 <b>CL</b>    | TMP4x 2796-<br>5 <b>CL</b>      | 28.0                                  | 33.<br>5 | 8.0   | 11       | 6.5   | 9.1 |
| TMP2x<br>2625-5 <b>HP</b>    | TMP4x 2796-<br>5 <b>HP</b>      | 35.0                                  | 35.<br>0 | 9.5   | 11.<br>5 | 7.8   | 9.3 |
| TMP2x<br>2625-5 <b>OP</b>    | TMP4x 2796-<br>5 <b>OP</b>      | 27.5                                  | 29.<br>5 | 8.5   | 8.5      | 6.6   | 7.0 |

| TMP2x             | TMP4x 4698- | 26.5 | 37. | 75   | 11. | 6.0  | 00  |
|-------------------|-------------|------|-----|------|-----|------|-----|
| 2829-62 <b>CL</b> | 1 <b>CL</b> | 20.3 | 0   | 7.5  | 0   | 0.0  | 0.0 |
| TMP2x             | TMP4x 4698- | 28.5 | 27. | 75   | 80  | 50   | 6.6 |
| 2829-62 <b>HP</b> | 1 <b>HP</b> | 20.5 | 5   | 1.5  | 0.0 | 5.0  | 0.0 |
| TMP2x             | TMP4x 4698- | 25.0 | 28. | 75   | 05  | 61   | 7.0 |
| 2829-62 <b>OP</b> | 1 <b>0P</b> | 25.0 | 5   | 7.5  | 0.5 | 0.1  | 7.0 |
| TMB2x             | TMP4x 6930- | 36.5 | 28. | 11.0 | 80  | 80   | 6.6 |
| 8084-2 <b>CL</b>  | 1 <b>CL</b> | 30.5 | 0   | 11.0 | 8.0 | 0.9  | 0.0 |
| TMB2x             | TMP4x 6930- | 33.0 | 33. | 10.5 | 11. | 81   | 0.0 |
| 8084-2 <b>HP</b>  | 1 <b>HP</b> | 55.0 | 0   | 10.5 | 0   | 0.1  | 9.0 |
| TMB2x             | TMP4x 6930- | 28.0 | 31. | 80   | 0.0 | 7.2  | 72  |
| 8084-2 <b>OP</b>  | 1 <b>0P</b> | 28.0 | 0   | 8.0  | 9.0 | 1.2  | 7.5 |
| TMB2x             | TMP4x 7002- | 20.0 | 27. | 0.0  | 85  | 74   | 7.0 |
| 8532-1 <b>CL</b>  | 1 <b>CL</b> | 29.0 | 5   | 9.0  | 0.5 | 7.4  | 7.0 |
| TMB2x             | TMP4x 7002- | 34.5 | 31  | 11.0 | 0.0 | 7.0  | 72  |
| 8532-1 <b>HP</b>  | 1 <b>HP</b> | 54.5 | 51  | 11.0 | 9.0 | 1.9  | 1,2 |
| TMB2x             | TMP4x 7002- | 30.0 | 29. | 85   | 0.0 | 6.5  | 75  |
| 8532-1 <b>OP</b>  | 1 <b>0P</b> | 30.0 | 0   | 0.5  | 9.0 | 0.5  | 1.5 |
| TMB2x             | TMP4x 7152- | 27.5 | 33. | 115  | 10. | 0.2  | 03  |
| 9839-3 <b>CL</b>  | 2CL         | 21.5 | 0   | 11.5 | 5   | 9.2  | 9.5 |
| TMB2x             | TMP4x 7152- | 32.0 | 33. | 11.5 | 11. | 0.8  | 0.0 |
| 9839-3 <b>HP</b>  | 2 <b>HP</b> | 32.0 | 5   | 11.5 | 0   | 9.0  | 9.0 |
| TMB2x             | TMP4x 7152- | 31.0 | 27. | 10.5 | 85  | 0.1  | 74  |
| 9839-3 <b>OP</b>  | 2 <b>0P</b> | 51.0 | 5   | 10.5 | 0.5 | 2.1  | 7.4 |
| LSD 0.05          |             | 7.79 |     | 2.53 |     | 2.39 |     |

Among diploids, this was the case between the hand pollinated TMB2x 8532-1HP and the open pollinated TMB2x 8532-1OP. With tetraploids, this was found between the hand pollinated TMP4x 2796-5HP and open pollinated TMP4x 2796-5OP and also between the hand pollinated TMP4x 7152-2HP and open pollinated TMP4x 7152-2OP. In these instances, the hand pollinated progenies performed better than their counterparts the open pollinated progenies. With regard to the number of youngest leaves showing necrotic spots at flowering (YSLF); in the diploids, the parental clone TMP2x 1448-1CL differed significantly only from its open pollinated progeny TMP2x 1448-1OP. There were no significant differences among diploids of similar genotype. With tetraploids TMP4x 2796-5HP differed significantly from TMP4x 2796-5OP but not from its parental clone, whereas TMP4x 6930-1HP differed from its parental clone TMP4x 6930-1CL but not its open pollinated counterpart.

Black Sigatoka Indices (BSI) and Leaf Retention Indices (LRI)

The open-pollinated diploid banana TMB2x 8084-2 OP displayed significantly higher (P=.05) black sigatoka resistance (BSI) than its hand pollinated sibling TMB2x 8084-2HP and its parental clone TMB2x 8084-2Cl (Table 3). However, their leaf retention indices (LRI) did not differ significantly (Table 3). Also the diploid banana parental clone TMB2x 8532-1Cl exhibited significantly higher black sigatoka resistance than its hand pollinated progeny TMB2x 8532-1HP but again their LRI did not differ significantly. Whereas the tetraploid plantain parental clone TMP4x 1658-4Cl showed significantly higher resistance to black sigatoka than its open pollinated progeny TMP4x 1658-4OP, their LRI did not differ significantly. The tetraploid plantain parental clone TMP4x 7152-2Cl had significantly higher BSI than its

hand pollinated progeny TMP4x 7152-2HP though their LRI again did not differ significantly. Thus for both banana and plantain genotypes there was no consistent pattern in resistance to black sigatoka when comparing parental clones and their hand or open pollinated progenies.

Table 3. Black Sigatoka Indices (BSI) and Leaf Retention Indices (LRI) of Parental clones (CL), hand pollinated (HP) and open pollinated (OP) diploid (2x) and tetraploid (4x) Banana & Plantain genotypes under naturally occurring infection by Black Sigatoka disease over 3 crop cycles at IITA High Rainfall Station, Onne, Rivers State, Nigeria

| Diploid (2x)            | Tetraploid                | В    | SI   | LRI  |      |  |
|-------------------------|---------------------------|------|------|------|------|--|
| Genotypes               | (4x)<br>Genotypes         | 2x   | 4x   | 2x   | 4x   |  |
| TMP2x 1448-1 <b>CL</b>  | TMP4x<br>1658-4 <b>CL</b> | 0.78 | 0.87 | 0.30 | 0.27 |  |
| TMP2x 1448-1 <b>HP</b>  | TMP4x<br>1658-4 <b>HP</b> | 0.77 | 0.81 | 0.33 | 0.27 |  |
| TMP2x 1448-1 <b>OP</b>  | TMP4x<br>1658-4 <b>OP</b> | 0.80 | 0.79 | 0.29 | 0.31 |  |
| TMP2x 2625-5 <b>CL</b>  | TMP4x<br>2796-5 <b>CL</b> | 0.81 | 0.83 | 0.29 | 0.33 |  |
| TMP2x 2625-5 <b>HP</b>  | TMP4x<br>2796-5 <b>HP</b> | 0.82 | 0.81 | 0.27 | 0.33 |  |
| TMP2x 2625-5 <b>OP</b>  | TMP4x<br>2796-5 <b>OP</b> | 0.78 | 0.82 | 0.31 | 0.29 |  |
| TMP2x 2829-62 <b>CL</b> | TMP4x<br>4698-1 <b>CL</b> | 0.80 | 0.80 | 0.28 | 0.30 |  |
| TMP2x 2829-62 <b>HP</b> | TMP4x<br>4698-1 <b>HP</b> | 0.77 | 0.83 | 0.26 | 0.29 |  |
| TMP2x 2829-62 <b>OP</b> | TMP4x<br>4698-1 <b>OP</b> | 0.81 | 0.82 | 0.30 | 0.30 |  |
| TMB2x 8084-2 <b>CL</b>  | TMP4x<br>6930-1 <b>CL</b> | 0.81 | 0.83 | 0.30 | 0.29 |  |
| TMB2x 8084-2 <b>HP</b>  | TMP4x<br>6930-1 <b>HP</b> | 0.77 | 0.82 | 0.32 | 0.33 |  |
| TMB2x 8084-2 <b>OP</b>  | TMP4x<br>6930-1 <b>OP</b> | 0.90 | 0.81 | 0.29 | 0.29 |  |
| TMB2x 8532-1 <b>CL</b>  | TMP4x<br>7002-1 <b>CL</b> | 0.82 | 0.82 | 0.31 | 0.31 |  |
| TMB2x 8532-1 <b>HP</b>  | TMP4x<br>7002-1 <b>HP</b> | 0.72 | 0.80 | 0.32 | 0.29 |  |
| TMB2x 8532-1 <b>OP</b>  | TMP4x<br>7002-1 <b>OP</b> | 0.76 | 0.83 | 0.28 | 0.31 |  |
| TMB2x 9839-3CL          | TMP4x<br>7152-2 <b>CL</b> | 0.80 | 0.89 | 0.42 | 0.32 |  |
| TMB2x 9839-3 <b>HP</b>  | TMP4x<br>7152-2 <b>HP</b> | 0.85 | 0.82 | 0.36 | 0.33 |  |
| TMB2x 9839-3 <b>OP</b>  | TMP4x<br>7152-2 <b>OP</b> | 0.87 | 0.87 | 0.34 | 0.31 |  |
| LSD 0.05                | 0.0                       | 080  | 0.0  | 55   |      |  |

**CL** = Parental clones; **HP** = Hand pollination; **OP** = Open pollination

In contrast, the diploid banana genotype TMB2x 9839 with numerically higher BSI did not show significant differences in BSI between parental clone and corresponding progeny but had significant differences (P=.05) in LRI between the parental clone and both progenies (Table 3). There were no significant differences between 4x genotypes for leaf retention index (LRI). Interploidy comparison showed that generally 4x-parental clones had numerically higher BSI than 2xparental clones thus expressing higher black sigatoka resistance than those of 2x- parental clones. There was no significant difference between 4x and 2x progenies for this trait. On average open pollinated 2x and 4x progenies had higher but not significantly different BSI than hand pollinated 2x and 4x progenies respectively. Host resistance as measured by black sigatoka index (BSI) depicted that this trait was higher in TMB2x 8084-2OP than TMB2x 8084-2HP and TMB2x 8084-2Cl. Higher resistance of this clone is related to its ability to slow symptom development and spread in the leaves. The better performance may be attributed to the pollination method. For the open pollinated genotypes, the chance of receiving different pollen mixtures may have resulted to improved disease resistance. Genetic improvement in Musa is targeted at obtaining progeny with improved disease resistance than their parental genotype. This was achieved in this study as TMB2x 8084-2OP had significantly higher BSI than the parental clone. Conversely, TMP4x 1658-4OP and TMP4x 7152-2HP had lower BSI than their respective parental clones. These differences can best be explained by additive gene action [38], [51].

### Vegetative Traits and Black Sigatoka Disease Indices

The responses of some vegetative traits of the plantain and banana genotypes under natural infection of black sigatoka disease are presented in Table 4. Generally, 4x genotypes were taller with their plant girths bigger than 2x genotypes attesting to the fact that 4x genotypes also had higher BSI and showed better resistance to black sigatoka disease under natural infection [52], [53]. Among the 2x genotypes, of particular interest were those that showed high BSI implying higher resistance to black sigatoka disease like the open pollinated banana progenies TMB2x 8084-2OP and TMB2x 9838-3. Interestingly both genotypes despite their higher BSI did not differ significantly (P=.05) in height, girth and height of tallest following sucker from their hand pollinated counterparts and parental clones. Correlation analysis showed that for diploids, plant height was negatively associated with BSI (r = -0.295), plant girth was negatively associated with BSI (r = -0.166) and height of tallest following sucker was also negatively correlated with BSI (r = -0.288). Considering 4x genotypes with the highest BSI, the parental clone of plantain TMP4x 1658-4CL had significantly (P> 0.05) taller plants with more robust girth than its hand and open pollinated progenies whereas the open pollinated plantain TMP4x 7152-2OP only had significantly taller plants with more robust girth than its hand pollinated counterpart. With respect to height of tallest following sucker, the parental clone TMP4x 1658-4CL was significantly (P=.05) taller than its hand pollinated progeny just as the open pollinated progeny TMP4x 7152-2OP was significantly taller than its hand pollinated counterpart. Correlation analysis showed that for tetraploids, plant height was positively and significantly associated with BSI (r = 0.502\*), plant girth was positively correlated with plant girth (r = 0.436) and height of tallest following sucker was significantly and positively associated with BSI ( $r = 0.534^*$ ). Correlation analysis showed very weak relationships between

LRI and BSI in both the diploids (r = 0.099) and the tetraploids (r = 0.020).

Table 4. Vegetative traits of parental clones (CL) and progenies of Parental clones (CL) hand pollinated (HP) and open pollinated (OP) diploid (2x) and tetraploid (4x) Banana & Plantain genotypes under naturally occurring infection by Black Sigatoka disease over 3 crop cycles at IITA High Rainfall Station, Onne, Rivers State, Nigeria

| Diploid<br>(2x)<br>Genotypes | Tetraploid<br>(4x)<br>Genotypes | Plant height<br>(cm) |                | Plant<br>(cr | girth<br>m)  | Height of<br>tallest<br>following<br>sucker (cm) |                |
|------------------------------|---------------------------------|----------------------|----------------|--------------|--------------|--|----------------|
| o en organo                  | ochotypes                       | 2x                   | 4x             | 2x           | 4x           | 2x   | 4x             |
| TMP2x                        | TMP4x                           | 286.2                | 347.7          | 42.6         | 59.9         | 251.9  | 260.0          |
| 1446-1CL<br>TMD2             | 1038-4CL                        |                      |                |              |              |  |                |
| 1448-1 <b>HP</b>             | 1658-4 <b>HP</b>                | 281.2                | 298.1          | 39.7         | 52.8         | 284.2  | 209.9          |
| TMP2x                        | TMP4x                           | 077.0                | 074.0          | 20.2         | 10.5         | 0.07.4   | 246.1          |
| 1448-1 <b>OP</b>             | 1658-4 <b>OP</b>                | 277.9                | 274.3          | 38.2         | 40.5         | 267.4  |                |
| TMP2x                        | TMP4x                           | 205.2                | 201.6          | 40.5         | 44.6         | 245 4  | 261.4          |
| 2625-5 <b>CL</b>             | 2796-5 <b>CL</b>                | 283.2                | 291.0          | 40.5         |              | 243.4  |                |
| TMP2x                        | TMP4x                           | 254.2                | 306.0          | 35.6         | 166          | 231.8  | 259.1          |
| 2625-5 <b>HP</b>             | 2796-5 <b>HP</b>                | 234.2                | 500.9          | 55.0         | 40.0         | 231.0  | 239.1          |
| TMP2x                        | TMP4x                           | 300.4                | 297.8          | 40.9         | 44.2         | 273.1  | 279.0          |
| 2625-5 <b>OP</b>             | 2796-5 <b>OP</b>                | 500.1                | 277.0          | 40.7         |              | 273.1  |                |
| TMP2x                        | TMP4x                           | 264.2                | 311.4<br>295.4 | 40.4<br>36.6 | 51.1<br>51.1 | 270.0  | 248.9<br>233.3 |
| 2829-62CL                    | 4698-1CL                        |                      |                |              |              |  |                |
| TMP2x<br>2829-62 <b>HP</b>   | TMP4x<br>4698-1 <b>HP</b>       | 260.3                |                |              |              | 238.3  |                |
| TMP2x                        | TMP4x                           |                      | 280.8          | 38.5         | 42.3         | 250.3  | 256.9          |
| 2829-62 <b>OP</b>            | 4698-1 <b>OP</b>                | 245.3                |                |              |              |  |                |
| TMB2x                        | TMP4x                           | 2747                 | 309.2          | 40.7         | 51.6         | 262.7  | 280.8          |
| 8084-2 <b>CL</b>             | 6930-1 <b>CL</b>                | 274.7                |                |              |              |  |                |
| TMB2x                        | TMP4x                           | 260.2                | 310.0          | 41.4         | 52.5         | 261.0  | 2747           |
| 8084-2 <b>HP</b>             | 6930-1 <b>HP</b>                | 209.2                |                |              |              | 201.9  | 274.7          |
| TMB2x                        | TMP4x                           | 2547                 | 305.8          | 39.7         | 50.4         | 235.4  | 254.6          |
| 8084-2 <b>OP</b>             | 6930-1 <b>OP</b>                | 201.7                | 505.0          | 57.1         | 50.1         | 200.1  |                |
| TMB2x                        | TMP4x                           | 256.1                | 342.9          | 39.7         | 56.9         | 263.6  | 243.2          |
| 8532-1CL                     | 7002-1CL                        |                      |                |              |              |  |                |
| TMB2x                        | TMP4x                           | 253.9                | 299.7          | 39.3         | 49.1         | 246.9  | 250.0          |
| 8532-1HP                     | 7002-1 <b>HP</b>                |                      |                |              |              |  |                |
| 8532-1 <b>OP</b>             | 7002-1 <b>OP</b>                | 273.9                | 303.3          | 41.3         | 47.0         | 234.9  | 265.4          |
| TMB2x                        | TMP4x                           |                      |                |              |              |  |                |
| 9839-3 <b>CL</b>             | 7152-2 <b>CL</b>                | 212.5                | 339.8          | 33.8         | 56.4         | 229.9  | 299.5          |
| TMB2x                        | TMP4x                           | 263.8                | 237 5          | 41.3         | 37.5         | 247.0  | 224.1          |
| 9839-3 <b>HP</b>             | 7152-2 <b>HP</b>                | 205.8                | 231.3          | 41.3         |              | 247.0  | 224.1          |
| TMB2x                        | TMP4x                           | 238.4                | 308.9          | 36.5         | 47.1         | 236.8  | 270.0          |
| 9839-3 <b>OP</b>             | 7152-2 <b>OP</b>                | 200.1                | 500.9          | 50.5         | 17.1         | 200.0  | 270.0          |
| LSD <sub>0.05</sub>          |                                 | 36.22                |                | 7.15         |              | 43.64  |                |

Yield components and Yields and Black Sigatoka Disease Indices

The responses of yield components and yields of the banana and plantain genotypes exposed to natural infection of black sigatoka disease in the field are presented in Table 5. The highest yielding diploid was the hand pollinated banana TMB2x 8084-2HP (5.2t/ha) followed by its open pollinated counterpart TMB2x 8084-2OP (4.6t/ha); both of them out yielding their parental clone (4.2t/ha). Whereas TMB2x 8084-2OP had exhibited the highest BSI, its hand pollinated counterpart TMB2x 8084-2HP had one of the lowest BSI though with a higher LRI than its open pollinated counterpart. This could have improved its yield potential as it had more photosynthetically active leaves. Others [46] had reported earlier that full-sibs could exhibit distinct host responses to black sigatoka disease. The third highest yielding diploid was the hand pollinated banana TMB2x 8532-1HP. All three plants also had the highest bunch weights amongst diploids. Considering the tetraploids, the highest yielding were the parental clones TMP4x 7002-1CL (10.4t/ha) with its open pollinated progeny producing less than a third of its yield (2.8t/ha) and its hand pollinated progeny producing slightly more than half its yield (6.9t/ha). The second highest yielding tetraploid was the parental clone TMP4x 1658-4CL (8.1t/ha) with its progenies producing less than half its yield. Both parental clones had moderate to high BSI. Surprisingly the tetraploid parental clone with the highest BSI had the third highest yield of 7.6t/ha with its progenies producing only half its yield despite having moderate to high BSI.

Table 5. Yield components and yields of parental clones (CL) and progenies of Parental clones (CL) hand pollinated (HP) and pen pollinated (OP) diploid (2x) and tetraploid (4x) Banana & Plantain genotypes under naturally occurring infection by Black Sigatoka disease over 3 crop cycles at IITA High Rainfall Station, Onne, Rivers State, Nigeria

| Diploid (2x) Genotypes  | Tetraploid (4x)        | Bunch weight (kg) |     | Number of hands per<br>bunch |     | Number of fingers per<br>bunch |       | Yield (tons/ha) |      |
|-------------------------|------------------------|-------------------|-----|------------------------------|-----|--------------------------------|-------|-----------------|------|
|                         | Genotypes              | 2x                | 4x  | 2x                           | 4x  | 2x                             | 4x    | 2x              | 4x   |
| TMP2x 1448-1 <b>CL</b>  | TMP4x 1658-4 <b>CL</b> | 2.0               | 7.0 | 7.0                          | 6.3 | 113.0                          | 99.5  | 2.4             | 8.1  |
| TMP2x 1448-1 <b>HP</b>  | TMP4x 1658-4 <b>HP</b> | 2.7               | 2.9 | 6.2                          | 5.2 | 94.0                           | 76.3  | 3.7             | 3.0  |
| TMP2x 1448-1 <b>OP</b>  | TMP4x 1658-4 <b>OP</b> | 3.2               | 2.7 | 7.0                          | 6.3 | 118.2                          | 85.0  | 3.7             | 3.6  |
| TMP2x 2625-5CL          | TMP4x 2796-5 <b>CL</b> | 2.2               | 3.4 | 6.7                          | 6.2 | 107.0                          | 92.5  | 2.6             | 4.1  |
| TMP2x 2625-5 <b>HP</b>  | TMP4x 2796-5 <b>HP</b> | 2.1               | 3.2 | 6.8                          | 6.0 | 100.0                          | 93.0  | 2.5             | 3.7  |
| TMP2x 2625-5 <b>OP</b>  | TMP4x 2796-5 <b>OP</b> | 2.3               | 4.8 | 7.2                          | 7.0 | 118.0                          | 118.8 | 2.3             | 5.0  |
| TMP2x 2829-62 <b>CL</b> | TMP4x 4698-1 <b>CL</b> | 2.8               | 3.1 | 6.3                          | 6.5 | 100.8                          | 97.5  | 3.5             | 3.8  |
| TMP2x 2829-62 <b>HP</b> | TMP4x 4698-1 <b>HP</b> | 1.9               | 3.6 | 4.7                          | 6.7 | 72.3                           | 94.7  | 2.0             | 4.4  |
| TMP2x 2829-62 <b>OP</b> | TMP4x 4698-1 <b>OP</b> | 1.5               | 3.9 | 6.2                          | 7.5 | 102.8                          | 101.2 | 2.0             | 3.7  |
| TMB2x 8084-2 <b>CL</b>  | TMP4x 6930-1 <b>CL</b> | 3.8               | 3.9 | 7.7                          | 7.2 | 129.5                          | 106.0 | 4.2             | 4.5  |
| TMB2x 8084-2 <b>HP</b>  | TMP4x 6930-1 <b>HP</b> | 4.6               | 4.4 | 8.0                          | 7.5 | 134.0                          | 123.3 | 5.2             | 5.0  |
| TMB2x 8084-2 <b>OP</b>  | TMP4x 6930-1 <b>OP</b> | 3.9               | 3.6 | 7.8                          | 6.7 | 140.2                          | 111.8 | 4.6             | 3.9  |
| TMB2x 8532-1 <b>CL</b>  | TMP4x 7002-1 <b>CL</b> | 3.3               | 7.9 | 7.2                          | 6.8 | 123.7                          | 103.8 | 3.7             | 10.4 |
| TMB2x 8532-1 <b>HP</b>  | TMP4x 7002-1 <b>HP</b> | 4.1               | 2.3 | 7.0                          | 5.3 | 120.3                          | 77.2  | 4.4             | 2.8  |
| TMB2x 8532-1 <b>OP</b>  | TMP4x 7002-1 <b>OP</b> | 2.7               | 5.2 | 6.2                          | 5.7 | 106.2                          | 85.0  | 3.2             | 6.9  |
| TMB2x 9839-3CL          | TMP4x 7152-2 <b>CL</b> | 2.6               | 6.8 | 5.8                          | 7.3 | 87.8                           | 101.5 | 3.7             | 7.6  |
| TMB2x 9839-3HP          | TMP4x 7152-2 <b>HP</b> | 1.7               | 2.8 | 5.8                          | 6.7 | 90.0                           | 92.5  | 2.4             | 3.3  |
| TMB2x 9839-3 <b>OP</b>  | TMP4x 7152-2 <b>OP</b> | 2.0               | 3.0 | 6.3                          | 6.3 | 112.5                          | 88.8  | 2.3             | 3.6  |
| LSD <sub>0.05</sub>     |                        | 2.1               | 39  | 1.                           | 39  | 27.18                          |       | 2.58            |      |

On average, bunch weight, and yields of tetraploids were higher than those of diploids under naturally occurring black sigatoka infection. Similar findings have been reported by [54]. In diploids bunch weight was negatively correlated with BSI (r = -0.172) whereas in tetraploids bunch weight was positively and significantly associated with BSI (r = 0.551\*). Other yield components showed weak correlations with BSI in both diploids (Number of hands per bunch and BSI r = 0.123; fingers per bunch and BSI r = 0.192) and tetraploids (Number of hands per bunch and BSI r = 0.261; fingers per bunch and BSI, r = 0.133). With respect to final yields, there was a negative relationship between yield and BSI in diploids (r = -0.112), whereas there was a significant positive association between yield and BSI (r = 0.477\*) in tetraploids.

### IV. CONCLUSION

This experiment has shown that the open pollinated diploid TMB2x 8084-2OP and its counterpart the hand pollinated diploid TMB2x 8084-2HP have potential as black sigatoka resistance genotype producing fairly good yields under naturally occurring black sigatoka infection. Other high yielding tetraploid genotypes with good black sigatoka

resistance include the parental clones TMP4x 7002-1CL, TMP4x 1658-4CL and TMP4x 7152-2CL.

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#### REFERENCES

- [1] Castelan, F.P., Saraiva, L.A., Lange, F., de Lapeyre de Bellaire, L., Cordenunsi, B.R., Chillet, M., (2012). Effects of black leaf streak disease and Sigatoka disease on fruit quality and maturation process of bananas produced in the subtropical conditions of southern Brazil. *Crop Prot.* 35, 127–131. https://doi.org/10.1016/j.cropro.2011.08.002
- [2] Dita M, Garming H, Van den Bergh I, Staver C, Lescot T. (2013). Banana in Latin America and the Caribbean: current state, challenges and perspectives. Act Hort. 986:365–80. https://doi.org/10.17660/ ActaHortic.2013.986.39
- [3] Zandjanakou-Tachin, M., Ojiambo, P.S., Vroh-Bi, I., Tenkouano, A., Gumedzoe, Y.M., Bandyopadhyay, R., (2013). Pathogenic variation of *Mycosphaerella* species infecting banana and plantain in Nigeria. *Plant Pathol.* 62, 298–308. https://doi.org/10.1111/j.1365-3059.2012.02650.x
- [4] Meredith, D.S., Lawrence J.S. Black leaf streak disease of bananas (*Mycosphaerella fijiensis*): Symptoms of disease in Hawaii, and

notes on the conidial state of the causal fungus. *Trans. Br. Mycol.* Soc. 1969:52, 459–476.

- [5] Rhodes, P.L. (1964). A new banana disease in Fiji. Commonwealth Phytopathological News. 10: 38-40
- [6] Carlier J, Foure' E, Gauhl F, Jones DR, (2000). Lepoivre P, Mourichon X, et al. Fungal diseases of the foliage: Sigatoka leaf spots. In: Jones DR, editor. Diseases of Banana, Abaca' and Enset. Wallingford, Oxon OX10 8DE UK: CABI Publishing;
- [7] Henderson J, Pattemore J, Porchun S, Hayden H, Van Brunschot S, Grice K, *et al.* (2006). Black Sigatoka disease: new technologies to strengthen eradication strategies in Australia. Australas Plant Pathol.; 35(2):181–93.
- [8] Ioos R, Hubert J, Abadie C, Dufe´ al D, Opdebeeck G, Iotti J. (2011). First report of black Sigatoka disease in banana caused by *Mycosphaerella fijiensis* on Martinique Island. Plant Dis. 95(3):359.
- [9] Robert S, Ravigne V, Zapater MF, Abadie C, Carlier J. (2012). Contrasting introduction scenarios among continents in the worldwide invasion of the banana fungal pathogen *Mycosphaerella fijiensis*. Mol Ecol. 21(5):1098–114. https://doi.org/10.1111/j.1365-294X.2011.05432.x PMID: 22256778
- [10] Brito, F.S.D., Fraaije, B., Miller, R.N.G., (2015). Sigatoka disease complex of banana in Brazil: Management practices and future directions. *Outlooks Pest Manag.* 26, 78–81. https://doi.org/10.1564/v26\_apr\_08
- [11] Frossard, P. (1980). Apparition d'une nouvelle et grave maladie foliare de bananiers et plantains au Gabon: le maladie de raies noires *Mycosphaerella fijiensis* Morelet. Fruits. 53: 519-527.
- [12] Fouré É. (1985). Les cercosporioses du bananier et leurs traitements. Etude de la sensibilité varietale des bananiers et plantains á *Mycosphaerella fijiensis* Morelet au Gabon (maladie des raies noires). III: Comportement des variétés. *Fruits* 40, 393– 399.
- [13] Bebber DP. (2019). Climate change effects on Black Sigatoka disease of banana. Phil. Trans. R. Soc. B. 374. 20180269. http://dx.doi.org/10.1098/rstb.2018.0269
- [14] Jesus Junior W. C; R. Valadares Júnior; R A Cecílio; W B Moraes; F Xavier Ribeiro do Vale; F. Ramos Alves; P A Paul. (2008). Worldwide geographical distribution of Black sigatoka for banana: predictions based on climate change models. Sci. Agric. (Piracicaba, Braz.), v.65, special issue. 40-53,
- [15] Rieux A. B. Hostachy, L. De Lapeyre de Bellaire, Y. Martin, G. Maratchia, A-S. Dupuis, R. Ioos, C. Jeandel and J. Hubert. (2019). First report of black Sigatoka disease in banana caused by *Mycosphaerella fijiensis* on Reunion Island. *New Disease Reports*. 39:12. http://dx.doi.org/10.5197/j.2044-0588.2019.039.012
- [16] EPPO (2014). Mycosphaerella fijiensis Paris, France: EPPO. Available from: https://gd.eppo.int/taxon/MYCOFI/distribution.
- [17] CABI (2015). Micosphaerella fijiensis (black Sigatoka) datasheet England: CABI; Available from: http://www.cabi.org/isc/datasheet/35278.
- [18] Yonow T, Ramirez-Villegas J, Abadie C, Darnell RE, Ota N, Kriticos DJ. (2019). Black Sigatoka in bananas: Ecoclimatic suitability and disease pressure assessments. PLoS ONE 14(8): e0220601. https://doi.org/10.1371/journal. pone.0220601
- [19] Biosecurity. (2008). Final Import Risk Analysis Report for the Importation of Cavendish Bananas from the Philippines, Part C. Canberra, Australia: Biosecurity Australia, Australia
- [20] Grice, K., Henderson, J., Pattison, T., Thomas, J., Vawdrey, L., Young, A. (2009). Chapter 5: Banana. In: *Diseases of Fruit Crops in Australia*, Cooke, T., Persley D., House S., eds . CSIRO Publishing Collingwood, VIC. 65-89.
- [21] Arzanlou, M., Groenewald, J.Z., Fullerton, R.A., Abeln, E.C.A., Carlier, J., Zapater, M.-F., Buddenhagen, I.W., Viljoen, A., Crous, P.W. (20:19). Multiple gene genealogies and phenotypic characters differentiate several novel species of *Mycosphaerella* and related anamorphs on banana. *Persoonia Mol. Phylogeny Evol. Fungi.* 37. https://doi.org/10.3767/003158508X302212
- [22] Queennsland Department of Agriculture and Fisheries. QDAF. (2011). Black Sigatoka.

- [23] New South Wales (NSW) Government DPI. (2013). Exotic pest alert: Black sigatoka. 1st, New South Wales Department of Primary Industries (No. Primefact 1250). Plant Biosecurity, Orange, Australia.
- [24] Friesen, TL. (2016). Combating the Sigatoka Disease Complex on Banana. In: John, M. McDowell, Editor Virginia Tech, UNITED PLoS Genet12(8): e1006234.doi:10.1371/journal.pgen.1006234.
- [25] Cedeño G, Suarez C, Vera D, Fadda C, Jarvis D, Santis P. (2017). Detección temprana de resistencia a Mycosphaerella fijiensis en genotipos locales de Musáceas en Ecuador. Scientia Agropecuaria. 8(1): 29 – 42
- [26] [26] Chong, P., (2016). Origin, versatility and distribution of azole fungicide resistance in the banana black Sigatoka pathogen *Pseudocercospora fijiensis*. Wageningen University, Wageningen, the Netherlands..
- [27] Marín D H; R A. Romero; M Guzmán; T B. Sutton. (2003). Black Sigatoka: An increasing threat to banana cultivation. Plant Disease. 87(3):208-222
- [28] Amil, A.F., Heaney, S.P., Stanger, C., Shaw, M.W. (2007). Dynamics of QoI sensitivity in *Mycosphaerella fijiensis* in Costa Rica during 2000 to 2003. *Phytopathology*. (97):1451–1457. https://doi.org/10.1094/PHYTO-97-11-1451
- [29] Cañas-Gutiérrez, G.P., Angarita-Velásquez, M.J., Restrepo-Flórez, J.M., Rodríguez, P., Moreno, C.X., Arango, R., (2009). Analysis of the CYP51 gene and encoded protein in propiconazole-resistant isolates of *Mycosphaerella fijiensis*. *Pest Manag. Sci.* 65:892–899. https://doi.org/10.1002/ps.1770
- [30] Torres L.C; LC Zamora. (2018). Benefits in Latin America and the Caribbean about production of Cavendish AAA banana resistant to black Sigatoka. 3(4):740-744
- [31] Stover R.H. (1990). Sigatoka leaf spots: Thirty years of changing control strategies: 1959-1989, in: Sigatoka leaf spot diseases of banana. International Network for the Improvement of Banana and Plantain, Montpellier, France. 66–74.
- [32] Jiménez MI. (2008). Effect of the nutritional status of banana (Musa spp.) on leaf disease infestation by Mycosphaerella fijiensis Morelet in Ecuador. Dissertation. Katholieke Universiteit Leuven, Belgium. 133.
- [33] Kablan L; Lagauche A; Delvaux B; Legrève A. (2012.. Silicon reduces black sigatoka development in banana. Plant Dis. 96(2):273–278.
- [34] Álvarez E; Cortés J; Ceballos G. (2010). Alternativas para el manejo de la Sigatoka negra en plátano Dominico Hartón (AAB) mediante el uso de lixiviado y productos biológicos. Bol MUSALAC. 1(2):3–5.
- [35] Emebiri, LC Oliefuna JC. (1992). Effects of leaf removal and intercropping on the incidence and severity of black sigatoka disease at the establishment phase of plantains *Musa* spp., AAB) Agriculture, Ecosystems and Environment. 39(3)213-210.
- [36] USDA, (2008). Peru, organic product update, USDA Foreign agricultural service. USA.
- [37] Fouré, E., Mouliom-Pefoura, A., Mourichon, X. (1990). Etude de la sensibilité variétale des bananiers plantains á *Mycosphaerella fijiensis* au Cameroun. Caractérisation de la résistance au champs de bananiers appartenant á drivers groupes génétiques. *Fruits* (45):329–338.
- [38] Ortiz, R., Vuylsteke, D. (1994). Inheritance of black Sigatoka disease resistance in plantain-banana (*Musa* spp.) hybrids. *Theor. Appl. Genet.* (89):146–152. https://doi.org/10.1007/BF00225134
- [39] Aguilar Moran, J. F. (2013). Improvement of Cavendish banana cultivars through conventional breeding. Acta Horticulturae. 2013;986:205–208. https://doi.org/10.17660/ActaHortic. 986.21
- [40] Amori m, E. P., Dos Santos-Serejo, J. A., Amorim, V., Ferreira, C. F., & Silva, S. O. (2013). Banana breeding at embrapa cassava and fruits. Acta Horticulturae. 986:171–176. https://doi.org/10.17660/ ActaHortic.2013.986.18
- [41] Wilson, M. (2015). Queensland biosecurity manual. Edition 2016, version 1. Biosecurity Queensland, Queensland Department of Agriculture and Fisheries..

- [42] Dadzie, B. K. and Orjeda, G. (1998). Post-harvest Characteristics of Black Sigatoka Resistant Banana, Cooking Banana and Plantain Hybrids. Inibap Technical Guidelines 4.;1-74.
- [43] Aïtchédji1, A. Tenkouano and O. Coulibaly (2010). Factors Affecting the Adoption of Disease-Resistant Plantain and Banana (*Musa* spp.) Hybrids in Nigeria. Proc. IC on Banana & Plantain in Africa. Eds.: T. Dubois *et al.* Acta Hort. 879, 741-748. ISHS
- [44] Ortiz R, Tenkouano A. (2011). Genotype by environment interaction and *Musa* Improvement. In M. Pillay & A. Tenkouano (Eds.), Banana breeding: Progress and Challenges (237–250 P). Boca Raton: CRC Press.
- [45] Ploetz, R.C., Kema, G.H.J., Ma, L.-J. (2015). Impact of diseases on export and smallholder production of banana. *Annu. Rev. Phytopathol.*,(53):269–288. https://doi.org/10.1146/annurev-phyto-080614-120305
- [46] Ortiz, R. Vuylsteke, D, Ferris, R. S. B., Okoro, J. U., N' Guessan, A., Hemeng, O. B., Yeboah, D K, Afreh-Nuamah, K. (1997). Developing new plantain varieties for Africa. Plant Var. Seeds. 10:39–57
- [47] Ortiz, R. Plot techniques for assessment of bunch weight in banana trials under two systems of crop management. Agron. J. 87:63–69.
- [48] Tenkouano, A., Oselebe, H. and Ortiz, R. (2010). Selection efficiency in *Musa* L. under different cropping systems. Austr. J. Crop Sci. 4:74–80

- [49] Swennen, R. (1990). Plantain cultivation under West African conditions: A reference manual. International Institute of Tropical Agriculture, Ibadan, Nigeria. 24.
- [50] Ortiz, R and D. (1995). Vuylsteke. Inheritance of dwarfism in plantain (*Musa* SPP., AAB group). Plant Breeding. 114: 466-468.
- [51] Craenen, K. and Ortiz, R. (1997). Effect of the *bs1* gene in plantain-banana hybrids on response to black Sigatoka. Theor. Appl. Genet. 95:497–505.
- [52] Vuylsteke, R.D; Swennen R and R. Ortiz. (1993). Development and performance of black sigatoka resistant tetraploid hybrids of plantain (Musa spp. AAB group). Euphytica 65:33-42.
- [53] Craenen, K. and Ortiz, R. (1998). Influence of black Sigatoka disease on the growth and yield of diploid and tetraploid hybrid plantains. Crop Protection. 17:13–18.
- [54] Tenkouano, A; N Lamien, J Agogbua, D Amah, R Swennen, S Traor'e, D Thiemele, N Aby, K Kobenan, G Gnonhouri, N Yao, G. Astin, S Sawadogo-Kabore, V Tarpaga, W Issa, B Lokossou, A Adjanohoun, G L Amadji, S Adangnitode, K A D Igue, and R Ortiz (2019). Promising High-Yielding Tetraploid Plantain-Bred Hybrids in West Africa. International Journal of Agronomy Volume 1-8. Article ID 3873198, https://doi.org/10.1155/2019/3873198