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Genotype X Environment Response of 'Matooke' Hybrids (Naritas) to *Pseudocercospora fijiensis*, the Cause of Black Sigatoka in Banana

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Abstract: Growing bananas resistant to *Pseudocercospora fijiensis*, the cause of black Sigatoka, is the preferred disease control strategy for resource-poor farmers. Banana breeding programs in east Africa have developed 27 Matooke hybrids (commonly known as NARITAs) with higher yields than local landraces. To assess the response of NARITA hybrids to *P. fijiensis*, 22 hybrids were evaluated under natural field conditions in four locations—Kawanda and Mbarara in Uganda, and Maruku, and Mitarula in Tanzania—between 2016 and 2018 for three crop cycles. Black Sigatoka was visually assessed and the area under the disease progress curve calculated for each plant over time. Significant differences ($p < 0.001$) were observed between genotypes, environments, and their interaction. The highest contributor to black Sigatoka severity (39.1%) was the environment, followed by the genotype (37.5%) and the genotype X environment interaction (GEI) (23.4%). NARITA 2, 7, 14, 21 and 23 were resistant and the most stable hybrids across locations. If other attributes such as the yield and taste are acceptable to end-users, these hybrids can be released to farmers in the region to replace highly susceptible landraces. Mitarula was identified as an ideal site for evaluating banana against black Sigatoka and should be used as a representative location to minimize costs of disease evaluations.

Keywords: Sigatoka; east African highland bananas (EAHB), NARITAs; stability; genotype by environment interaction (GEI)

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1. Introduction

Banana is an important staple crop in developing countries, especially in the tropics and sub-tropics, where it is ranked fourth in importance after wheat, rice and maize [1]. In Africa, banana is mostly produced by smallholder farmers, primarily for home consumption, while the surplus is sold in local and regional markets [2–4]. Banana provides up to a fifth of the total calorie intake in east and central Africa (ECA), with per capita consumption ranging between 250 and 600 kg annually [2]. The east African highland bananas (EAHB) include a genetically uniform triploid (AAA) group of cooking banana belonging to the 'Mutika-Lujugira' subgroup, called Matooke bananas [5–8] bananas are a product of hybridization between *Musa acuminata* ssp. *zebrina* and spp. *banksii*, with a contribution from *M. schizocarpa* [5,9]. Matooke are the preferred banana in Africa's Great Lakes region [10].

East African highland bananas account for 80% of all bananas produced in ECA [3]. However, productivity is low due to declining soil fertility, pests (weevils and nematodes) and diseases [11]. One of the most important diseases is black Sigatoka, a foliar disease caused by the ascomycetous fungus *Pseudocercospora fijiensis* (Morelet). The disease manifests as necrotic lesions that aggregate to cover entire leaf surfaces and reduce photosynthesis [12–14]. This results in small bunches with poorly filled fingers [13]. Yield losses can be over 50%, depending on the cultivar affected and the prevailing environmental conditions [13–15].

Black Sigatoka can be managed with fungicides [14,16] but this is not an option for smallholder farmers in Africa, especially because banana are grown close to the homestead, and also due to the high costs and limited availability of the chemicals. Furthermore, the use of fungicides is a risk to human health and is harmful to the environment [13,14]. Therefore, the use of resistant banana varieties is the most desirable and environmentally friendly approach. Resistant banana hybrids can be developed through conventional breeding [17–19], which is a slow process. It takes more than seven years to develop a banana hybrid and may take up to 17 years for a hybrid to be planted by farmers [19]. Banana breeding is complicated by low genetic variability, the limited production of viable seeds, female sterility and differences in ploidy levels [17,20,21]. Disease-resistant bananas can also be developed through genetic modification [22,23]. Transgenic plants with reduced black Sigatoka symptoms have been developed [24–26]. However, their cultivation has been hampered by the lack of regulations governing adoption of transgenic plants and opposition from governments and the public. Genome editing using CRISPR/Cas has emerged as a robust tool for improving crop traits like enhanced yields and tolerance to biotic and abiotic stresses [27,28]. This technology is yet to be applied in developing black Sigatoka resistance in banana. There are, however, biosafety concerns with genome-edited cops; thus, conventional breeding remains the widely acceptable strategy for developing disease-resistant bananas.

Matooke bananas are highly susceptible to black Sigatoka [29,30]. The development of black Sigatoka-resistant banana varieties, therefore, will greatly benefit smallholder farmers. The International Institute of Tropical Agriculture (IITA) and the National Agricultural Research Organisation (NARO) breeding programs in Uganda developed hybrids by crossing Matooke cultivars with Calcutta-4 (AA), which is a donor of black Sigatoka resistance [30,31]. The resulting tetraploids were then crossed with improved diploid hybrids derived from different parents to generate triploid hybrids known as NARITA bananas [21]. Tushemereirwe [30] evaluated 25 NARITA hybrids at Sendusu in Uganda and reported that they had higher yields and better resistance to black Sigatoka than 'Mbwazirume', a preferred local Matooke cultivar. However, these hybrids have not been evaluated in other locations.

The development and severity of black Sigatoka is dependent on prevailing environmental conditions [13,32], and the local *P. fijiensis* population [33]. It is, therefore, important to understand the effects of the environment (E), genotype (G), and their interactions (GEI), in response of the host to *P. fijiensis*. Such information can be obtained by evaluating hybrids across sites over multiple years. This information is useful for selecting hybrids that are stable and perform well across locations [33,34]. In this study, we evaluated 22 NARITA hybrids for response to black Sigatoka under four agro-ecologies in Uganda and Tanzania. These experiments were used to study the effect of GEI on hybrid stability and adaptability, and to identify an ideal test site in ECA to screen banana germplasm against *P. fijiensis*.

2. Materials and Methods

2.1. Planting Materials

Twenty-two NARITA hybrids were planted at two sites in Tanzania (Maruku, Mitarula in Kagera and Mbeya districts, respectively) and 17 hybrids in two test sites in Uganda, Kawanda and Mbarara (Table 1). At each test site, 10 subsamples were randomly collected

from each block at a depth of 0 to 20 cm. The subsamples were pooled and thoroughly mixed to make a composite sample per block. From each composite sample, 200 to 300 g of soil was placed in sample bags and oven dried for two to three days at 70 to 80 °C. All samples were then submitted to the Soil and Plant Analytical Laboratories at the National Agricultural Research Labs (NARL), Kawanda in Uganda for mineral analysis.

Table 1. Soil analysis and location of sites used for multi-location evaluation of Matooke hybrids (NARITAs) and banana varieties for black Sigatoka resistance evaluation.

Country	Location	Grid reference (Decimal Degrees) Longitude	Latitude	Altitude (masl)	Rainfall (mm)	Average Temperatures °C	pH	OM (%)	P (ppm)	Ca (ppm)	Mg (ppm)	K (ppm)	Soil Type
Uganda	Kawanda	0.414833	32.53238	1196	900–1500	>22	5.1	2.7	9.5	2516.9	611.0	341.4	Sandy clay
	Mbarara	−0.60032	30.59843	1412	900–1500	>22	4.7	1.8	11.0	1757.7	668.4	456.8	Sandy loam
Tanzania	Maruku	−1.42446	31.77358	1364	1500–2500	>22	5.3	3.0	18.0	1262.9	291.2	964.8	Clay loam
	Mitarula	−9.39769	33.62753	1055	>900	>22	4.6	7.1	4.2	361.3	47.1	176.8	Sandy loam

masl—meters above sea level; OM—organic matter; P—phosphorous content; Ca—calcium levels; Mg—magnesium content, K—potassium content.

Four susceptible banana varieties—Cavendish cv. Williams (AAA), Cachaco (Bluggoe, ABB), Gros Michel (AAA) and Mbwazirume (AAA)—were included as controls. Pisang Ceylan (Mysore, AAB), a black Sigatoka-resistant check, was only planted in Uganda. The trials were established using greenhouse-hardened tissue culture-derived plantlets obtained from KilimOrgano Limited in Dar es Salaam, Tanzania and BioCrops Limited in Kampala, Uganda. Due to challenges with tissue culture multiplication, only 19 cultivars (15 NARITA hybrids and four checks) were common across all sites (Supplementary Table S1). NARITA 19, 20, 25, 26 and 27 were only planted in Tanzania, while NARITA 24 was only planted in Uganda. NARITA 17 was only planted in Kawanda.

2.2. Field Trials

Trials were planted between March and May 2016. The experiments were laid out in a completely randomized block design, with four replications. Three-month-old plants were planted in 0.4 m deep and 0.6 m wide holes, with 3-m intra- and inter-row spacing. Before planting, 10 kg of decomposed cow manure was applied in each hole. Each accession was planted in two-row plots, with 12 plants per replication. The black Sigatoka-susceptible Cavendish cultivar Williams was planted as a guard row and spreader of *P. fijiensis* at all locations.

The trials were mulched with dry grass three months after planting. Weeding was carried out by hand. The trials were rainfed with no supplemental irrigation. No chemical or biological control products against black Sigatoka were applied. Weather data for the period of experiment was extracted from the World Weather Online website (https://www.worldweatheronline.com, accessed on 22 October 2019).

2.3. Black Sigatoka Confirmation

During the evaluations, infected leaf samples were randomly collected from different genotypes for pathogen isolation and identification. Fungal DNA was extracted directly from banana leaves and the presence of *P. fijiensis* confirmed using the species-specific MFActF/ACTR primer set, as previously described [35]. In addition, single-spore isolates of *P. fijiensis* were obtained using the ascospore-discharge method, and the pathogen identified using *P. fijiensis*-specific primers.

2.4. Disease Evaluations

Disease evaluations started six months after planting when the susceptibility check had developed black Sigatoka symptoms. Three plants per genotype per replication were randomly selected, tagged and evaluated every three months until the plant was harvested. For each mat, evaluations were performed on the mother (cycle 1), the daughter (cycle 2) and granddaughter (cycle 3) plants. Each of the cycle lasted 9 to 12 months, depending

on the cultivar. The number of standing leaves and the youngest leaf spotted (YLS) were recorded at flowering and used to calculate the index of non-spotted leaves (INSL). Black Sigatoka severity was scored visually by estimating the leaf area with symptoms for each standing leaf using the method of [36], where 0 = no visible symptoms, 1 = less than 1% of leaf area infected, 2 = 1 to 5% infected, 3 = 6 to 15% infected, 4 = 16 to 33% infected, 5 = 34 to 50% infected and 6 = 51 to 100% of leaf area infected (Figure 1).

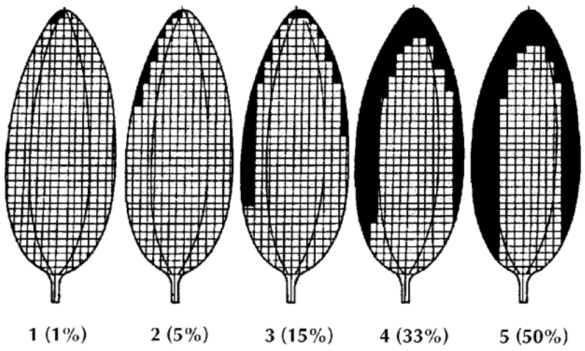

Figure 1. Sigatoka severity scoring. Adapted from [36].

2.5. Data Analysis

The index of non-spotted leaves was computed for each plant using the following formulae [37]:

$$\mathrm{INSL} = [(\mathrm{YLS} - 1) \div \mathrm{NSL}] \times 100$$

where:

YLS—rank of the youngest leaf counting from bottom bearing at least 10 necrotic lesions with a dry center,
NSL—the number of leaves starting from the youngest,
INSL—the proportion of standing leaves without the typical black Sigatoka late-stage necrotic lesions.

The black Sigatoka disease severity index for each plant was computed using the following formula [38]:

$$\mathrm{DSI} = \sum[n\mathrm{b} \div (\mathrm{N} - 1)\mathrm{T}] \times 100$$

where:

n = number of leaves in each disease severity group, b = % severity group; N = number of severity groups used in the scale (7); T = total number of leaves scored.

The disease severity index (DSI) at different evaluation times was used to calculate the area under the disease progress curve (AUDPC) [39], per cycle, using the formula:

$$\mathrm{AUDPC} = \sum_{ni} 1[(\mathrm{X_i} + 1 + \mathrm{X_i}) \div 2][\mathrm{t_i} + 1 - \mathrm{t_i}]$$

where $\mathrm{X_i}$ = disease severity index at ith day, $\mathrm{t_i}$ = the time in days after appearance of the disease at ith day, and n = the total number of observations.

A Pearson's correlation was performed to determine the association between Sigatoka evaluation parameters, mean DSI, YLS, INSL and AUDPC.

Analysis of variance (ANOVA) for disease scores was carried separately for each location to determine the effect of genotype, cycle, and genotype x cycle. For across-test-location (environments) comparison, only genotypes that were common across environments were included in the analysis. Multiple comparisons were performed using Fisher's least significant difference test ($p < 0.05$).

An additive main effect and multiplicative interaction (AMMI) model was used for GEI analysis using genotypes that were common across environments. The relationship among test environments, genotypes and GEIs, genotype main effects and genotype envi-

ronment (GGE) was visualized using biplots generated from plotting the first two principal components (PC1 and PC2) derived from single value decomposition of environmental data [40–42]. The stability of a genotype to black Sigatoka infection was visualized using the average environment coordinate (AEC) axis [41]. The AEC is plotted by taking the mean of PC1 and PC2 in all environments. A performance line passing through the origin of the biplot is used to determine the mean performance of the genotype. The stability of each genotype was shown by its projection onto the line drawn through the average environment and the biplot origin, the average environment axis (AEA; *X*-axis).

A regression analysis was performed to determine the influence of different weather variables—rainfall, minimum temperature, maximum temperature and relative humidity—on disease severity. All the analyses were implemented in GenStat software version19 and XLSTAT version 2019.

3. Results

3.1. Test Locations Characteristics

Soils at all test locations were sandy, except at Maruku, where the soils were of clay type. The highest organic matter content was reported at Mitarula (7.1%), while the lowest was reported at Mbarara (1.8%) (Table 1). Maruku had the highest amounts of phosphorous and potassium at 18.0 ppm and 964.8 ppm, respectively, while Maruku had the least at 4.2 and 176.8 ppm (Table 1).

3.2. Black Sigatoka Confirmation

DNA extracted from leaves with black Sigatoka symptoms in Kawanda, Mbarara, Mitarula and Maruku amplified a 500-bp fragment using *P. fijiensis*-specific primers, confirming that the leaf spot symptoms were caused by *P. fijiensis*.

3.3. Black Sigatoka Severity Evaluation Parameters

A significant positive correlation was obtained between the AUDPC and DSI (r = 0.83) and INSL and YLS (r = 0.60), while a significant negative correlation was observed between DSI and INSL (r = −0.85), AUDPC and INSL (r = −0.66) and AUDPC and YLS (r = −0.49) (Table 2). AUDPC had the highest coefficient of determination (R^2 = 0.87), followed by DSI (R^2 = 0.86) and INSL (R^2 = 0.84), while YLS had the lowest (R^2 = 0.78). AUDPC was thus used for further analysis.

Table 2. Correlation matrix and coefficient of determination of parameters used to evaluate Matooke hybrids (NARITAs) and banana varieties for response to black Sigatoka.

Disease Parameter	DSI [a]	AUDPC [b]	INSL [c]	YLS [d]	R^2 (Coefficient of Determination)
DSI	1				0.86
AUDPC	0.83 *	1			0.87
INSL	−0.85 *	−0.66 *	1		0.84
YLS	−0.57 *	−0.49	0.60 *	1	0.78

* Indicate significant correlation ($p < 0.001$). [a] Disease severity index. [b] Area under disease progress curve. [c] Index of non-spotted leaves. [d] Youngest leaf spotted.

3.4. Genotype Response at Each Crop Cycle

Disease severity differed significantly ($p < 0.001$) among banana genotypes and cycles at all sites. More disease was recorded in cycle 2 at all evaluation sites, and the mean AUDPC was 261.8 at Kawanda, 195.4 at Mbarara, 111.7 at Maruku and 135.2 at Mitarula. The lowest mean AUDPC was observed in cycle 1 at all locations except Maruku, where cycle 3 plants developed the least disease symptoms (Table 3).

The genotype X cycle interaction led to an inconsistent genotype ranking at the same location. Due to the higher and consistent disease severity in cycle 2 across the testing sites, data from this cycle was used for genotype ranking.

Table 3. Mean black Sigatoka severity (AUDPC) of Matooke hybrids (NARITAs) and banana varieties at each of the four locations in Uganda and Tanzania between 2016 and 2018. Means were compared between cycles at each test location.

	Area Under Disease Progress Curve (AUDPC) *											
	Kawanda			Mbarara			Maruku			Mitarula		
Genotype	Cycle 1	Cycle 2	Cycle 3	Cycle 1	Cycle 2	Cycle 3	Cycle 1	Cycle 2	Cycle 3	Cycle 1	Cycle 2	Cycle 3
NARITA 2	175.8 a	197.6 a	175.8 a	23.4 a	140.4 b	91.7 b	94.1 b	93.4 b	34.9 a	80.4 a	62.3 a	66.1 a
NARITA 4	101.3 a	126.7 a	133.1 a	33.7 a	136.1 b	96.7 b	78.8 a	131.9 b	51.4 a	59.2 a	65.0 a	71.3 a
NARITA 6	219.8 a	279.0 b	201.6 a	62.4 a	93.7 a	78.0 a	149.7 b	154.9 b	87.2 a	72.3 a	130.1 b	78.6 a
NARITA 7	142.2 a	240.8 b	205.8 b	41.2 a	166.2 b	152.0 b	80.7 a	165.8 b	49.9 a	84.8 a	115.8 ab	102.5 a
NARITA 8	172.6 a	164.9 a	146.4 a	18.4 a	161.4 c	84.4 b	78.8 ab	104.15 b	45.7 a	71.4 a	95.4 a	77.1 a
NARITA 10	229.1 a	313.2 b	258.8 a	58.8 a	330.5 c	208.8 b	74.7 a	129.5 b	39.4 a	79.4 a	202.3 c	152.1 b
NARITA 11	187.6 a	311.2 c	249.2 b	23.9 a	147.8 c	90.6 b	107.1 b	133.0 b	57.7 a	96.4 a	122.6 ab	133.7 b
NARITA 12	220.1 a	384.8 c	293.4 b	35.3 a	185.5 b	175.2 b	34.5 ab	70.48 b	27.0 a	67.5 a	147.9 b	64.3 a
NARITA 13	152.0 a	419.0 c	298.1 b	44.4 a	190.7 c	96.5 b	68.8 a	144.6 b	53.5 a	113.1 a	190.9 c	126.8 b
NARITA 14	139.6 a	170.3 a	162.1 a	24.6 a	133.9 b	101.6 b	106.4 b	165.7 c	65.2 a	65.2 a	76.3 a	79.7 a
NARITA 15	312.1 b	333.3 b	206.9 a	43.8 a	187.0 b	176.1 b	35.7 a	68.8 a	35.0 a	112.4 a	163.3 b	107.7 a
NARITA 18	259.5 b	285.7 b	188.4 a	63.2 a	248.9 b	105.5 a	33.1 a	79.5 b	38.4 a	79.8 a	179 b	161.5 b
NARITA 21	167.1 a	199.3 a	187.7 a	30.2 a	152.1 b	137.7 b	102.2 ab	70.9 a	42.4 a	38.7 a	100.4 a	94.1 a
NARITA 22	142.9 a	157.3 a	178.6 a	27.2 a	155.9 b	113.3 b	59.4 a	100.2 b	39.3 a	42.4 a	89.1 ab	116.2 b
NARITA 23	197.7 a	210.6 a	165.8 a	21.3 a	190.2 b	148.8 b	50.5 a	50.6 a	26.3 a	39.1 a	125.2 b	61.4 a
Mbwazirume	189.6 a	266.7 b	259.4 b	79.4 a	253.1 c	150.8 b	43.0 a	77.3 a	46.9 a	86.8 a	175.8 b	150.6 b
Williams	250.1 a	320.6 b	303.8 b	73.0 a	317.8 c	157.8 b	138.3 b	184.9 c	93.4 a	82.7 a	200.6 b	180.7 b
Cachaco	244.7 a	349.2 b	286.7 a	59.8 a	324.1 c	222.6 b	59.5 a	105.8 b	36.9 a	72.5 a	132 b	121.5 b
Gros Michel	174.7 a	244.8 b	253.5 b	52.4 a	197.7 b	151.0 b	54.4 ab	84.2 b	34.2 a	81.4 a	195.7 b	156.7 b
Average	193.6 a	261.8 c	218.7 b	43.0 a	195.4 c	133.6 b	75.8 b	111.7 c	49.6 a	81.1 a	135.2 c	110.7 b

* Genotype mean (between the three cycles in a trial location) followed by the same letter do not differ significantly ($p < 0.05$).

3.5. Genotype Response to Black Sigatoka at Each Environment

Response of cultivars to black Sigatoka differed significantly at all sites (Table 4). Overall AUDPC was highest at Kawanda, ranging from 126.8 to 419.0 with a mean of 261.8, and the lowest at Maruku, ranging from 50.6 to 184.9 with a mean of 111.7 (Table 4).

Table 4. Disease severity (AUDPC) of black Sigatoka on Matooke hybrids (NARITAs) and banana varieties evaluated at four different locations in Uganda and Tanzania during cycle 2.

Genotype	Kawanda *	Mbarara *	Maruku *	Mitarula *	Uganda *	Tanzania *
NARITA 2	197.6 b–e (6)	140.4 a–d (6)	68.8 a–c (3)	62.3 a (1)	169.0 a–d (5)	65.4 ab (2)
NARITA 4	126.8 a (1)	136.1 a–c (4)	50.6 a (1)	65.0 ab (2)	132.0 a (1)	58.1 a (1)
NARITA 6	279.0 g–k (13)	93.7 a (1)	105.8 d–h (13)	130.1 e–I (13)	242.0 c–f (10)	117.4 e–I (15)
NARITA 7	240.8 b–h (9)	166.2 b–h (11)	84.1 b–f (9)	115.8 c–f (10)	203.5 c–e (9)	100 d–h (10)
NARITA 8	164.9 a–c (3)	161.4 b–h (10)	77.2 a–e (7)	95.4 a–e (8)	163.5 a–d (4)	86.3 b–f (7)
NARITA 10	314.0 j–m (18)	330.5 k (22)	165.8 j–m (23)	202.3 kl (24)	315.1 f–m (21)	190.1 m–o (23)
NARITA 11	311.4 i–m (17)	147.8 a–e (7)	104.2 c–h (12)	122.6 d–g (11)	275.0 f–l (16)	116.1 e–I (12)
NARITA 12	384.9 no (22)	185.5 e–h (12)	129.5 g–j (16)	147.9 f–j (17)	285.2 f–l (17)	137.9 i–k (17)
NARITA 13	419.0 o (23)	190.7 e–h (13)	133.0 h–k (18)	190.9 jk (21)	293.2 f–m (18)	163.1 j–m (19)
NARITA 14	170.4 a–c (4)	133.9 a–c (3)	70.5 a–c (4)	76.3 ab (3)	152.0 ab (2)	73.3 a–c (4)
NARITA 15	333.3 j–n (20)	187.0 e–h (14)	144.6 i–l (19)	163.3 g–j (18)	268.7 f–k (15)	153.1 j–l (18)
NARITA 16	296.2 h–m (15)	137.9 a–d (5)	_	124.7 d–h (12)	296.2 f–m (19)	125.4 c–j (16)
NARITA 17	245.1 b–j (11)	_	_	_	245.1 c–h (12)	_
NARITA 18	284.4 h–l (14)	248.9 gi (17)	165.7 j–m (22)	179.0 h–k (20)	262.2 f–I (13)	172.4 j–o (21)
NARITA 19	_	_	153.6 i–l (20)	232.1 l (25)	_	198.1 o (25)
NARITA 20	_	_	109.6 d–h (14)	127.2 e–I (14)	_	117.2 e–I (14)
NARITA 21	197.2 b–f (7)	152.1 a–f (8)	79.4 a–e (8)	100.4 b–e (9)	169.9 b–d (6)	92 c–g (8)
NARITA 22	157.3 ab (2)	155.9 b–g (9)	71 a–d (5)	89.1 a–e (7)	156.9 a–c (3)	83.1 a–e (6)
NARITA 23	210.7 b–g (8)	190.2 e–h (15)	100.2 c–g (11)	125.2 e–I (15)	200.4 c–e (8)	112.7 e–I (11)
NARITA 24	297.7 i–m (16)	295.3 jk (19)			297.7 f–m (20)	_
NARITA 25	_	_	73.8 a–d (6)	78.4 a–c (4)	_	74.8 a–d (5)
NARITA 26	_	_	60.5 ab (2)	84.2 a–d (5)	_	71.5 a–c (30
NARITA 27	_	_	120.5 g–I (15)	86.7 a–d (6)	_	97.5 c–h (9)
Mbwazirume	266.7 g–j (12)	253.1 g–j (18)	154.9 i–m (21)	175.8 h–k (19)	266.7 f–j (14)	166.5 j–n (20)
Williams	320.6 j–m (19)	317.8 k (20)	184.9 m (24)	200.6 j–l (23)	319.2 h–m (22)	192.1 no (24)
Pisang Ceylan	192.0 b–d (5)	128.3 ab (2)			192.0 b–e (7)	_
Cachaco	349.2 k–o (21)	324.1 k (21)	93.4 c–g (10)	132.0 e–I (16)	349.2 m (23)	116.6 e–I (13)
Gros Michel	244.8 b–j (10)	197.7 e–h (16)	131.9 g–j (17)	195.7 j–l (22)	244.8 e–g (11)	176.9 j–o (22)

* Means followed by the same letter in each column do not differ significantly $p > 0.05$ and number in parentheses indicate the relative genotype ranking at each test location and country.

The best performing hybrids at Kawanda were NARITA 2, 4, 8, 14 and 22, while the most susceptible ones were NARITA 12, 13 and 15 (Table 4). In Mbarara, the best performing hybrids were NARITA 2, 4, 6, 14 and 16, and their response to *P. fijiensis* did not differ significantly from the resistant check, Pisang Ceylan.

The most susceptible hybrids in Mbarara were NARITA 10, 18 and 24. At Maruku, NARITA 2, 4, 14, 22 and 26 developed the least leaf lesions and NARITA 10, 18 and 19 the most. Similarly, at Mitarula, NARITA 2, 4, 14, 25 and 26 performed best, and NARITA 10, 13 and 19 the worst (Table 4). Most of the hybrids were ranked similarly across sites, except for NARITA 6, which was the best hybrid in Mbarara and not at other locations (Table 4).

3.6. Genotype Response between Countries

Disease severity differed significantly between the sites in Uganda and Tanzania. The five best performing hybrids in Uganda were NARITA 2, 4, 8, 14 and 22, while the most susceptible hybrids were NARITA 10, 16 and 24. In Tanzania, the five best performing hybrids were NARITA 2, 4, 14, 25 and 26, and the most susceptible ones were NARITA 10, 18 and 19 (Table 4).

3.7. Multi-Location Analysis of NARITA Hybrids

AMMI analysis revealed that genotype, environment and GEI significantly ($p < 0.001$) influenced black Sigatoka severity. Most of the variation observed among hybrids was ascribed to the environment (39.1%), while the genotypes accounted for 37.5% and GEI for 23.4% of the variation (Table 5).

Table 5. Additive main effects and multiplicative interaction (AMMI) analysis of variance of black Sigatoka severity (AUDPC) of Matooke hybrids (NARITAs) and banana varieties evaluated in four environments of Uganda and Tanzania.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Sum of SQUARES	% Variance Explained
Treatments	75	2,277,733	30,370 *	-
Genotypes	18	854,825	47,490 *	37.5
Environments	3	890,810	296,937 *	39.1
Block	12	61,113	5093 ns	-
Interactions	54	532,098	9854 *	23.4
IPCA 1	20	263,413	13,171 *	49.5
IPCA 2	18	226,642	12,591 *	42.6
Residuals	16	42,044	2628 ns	-
Error	216	819,161	3792	-
Total	303	3,158,007	10,422	-

* Values are significantly different $p < 0.001$; ns—not statistically different.

The first and second interaction principal components (IPCA) axes were significant ($p < 0.001$), explaining 92.1% of the interaction sum of squares. IPCA1 and IPCA2 accounted for 49.5% and 42.6% of the total GEI sum of squares, respectively (Table 5).

The polygon view of the genotypes in the GGE biplot for 19 genotypes (15 NARITA hybrids and four checks) based on environment scaling showed that the first two principal components PC1 and PC2 were significant and explained 74.52% and 18.95%, respectively, representing 93.48% of the genotype and environment interaction. A polygon was formed by connecting the vertex genotypes with straight lines and the rest of the genotypes placed within the polygon (Figure 2).

The vertex genotypes placed farthest from the biplot origin were NARITA 4, 6, 10 and 13 (Figure 2). They represent either the most resistant or susceptible genotypes in all or some environments and contributed the most to the observed genotype by environment interaction. For example, NARITA 4 and 6 were the least susceptible to Sigatoka, while NARITA 10 and 13 had the highest disease severity. The test environments fell under three sectors with two corner cultivars. The first sector contains Mbarara, with NARITA 10 as the

most responsive hybrid; Maruku and Mitarula made up the second sector, while Kawanda fell under the third sector, with NARITA13 as the most responsive (Figure 2).

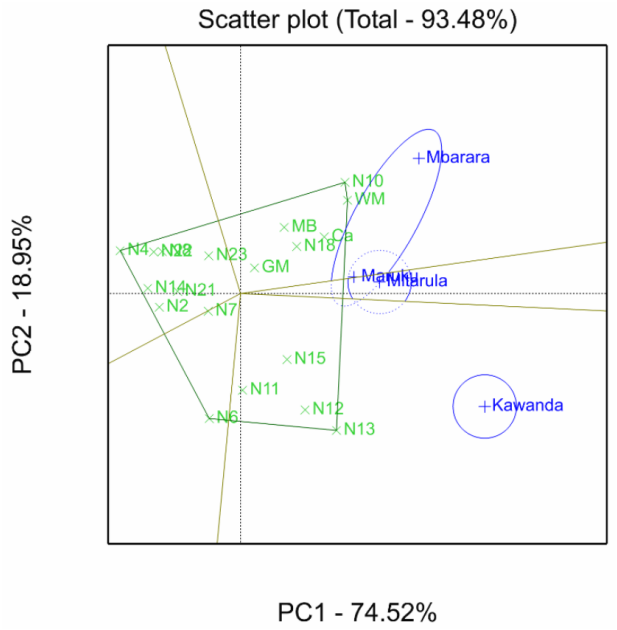

Figure 2. Polygon view of the genotype main effect and genotype x environment interaction biplot analysis for Sigatoka severity based on 19 banana cultivars evaluated in Uganda and Tanzania. Genotypes are given as codes (Matooke hybrids N—NARITA and banana varieties; MB—Mbwazirume, WM—Williams, Ca—Cachaco, GM—Gros Michel).

Ranking of genotypes based on both the mean disease severity and stability performance identified 10 hybrids with low mean disease severity (Figure 3).

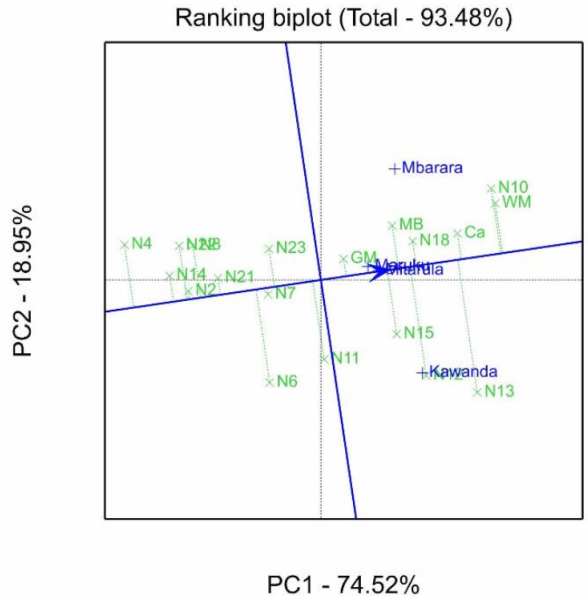

Figure 3. The "mean vs. stability" view of the GGE biplot showing the ranking of 19 banana genotypes (Matooke hybrids N—NARITA and banana varieties; MB—Mbwazirume, WM—Williams, Ca—Cachaco, GM—Gros Michel) evaluated in four locations for response to black Sigatoka (AUDPC) in 2016 to 2018. The 10 hybrids on the left side of the biplot developed the least disease, and those on the right-hand side the most disease. The most stable genotypes are those with a short projection on either side on the average environment axis (line with an arrow passing through the biplot origin).

The NARITAs with the least disease were 2, 4, 6, 7, 8, 11, 14, 21, 22 and 23. The most susceptible hybrids were NARITA 10, 12, 13, 15 and 18, and they clustered with the susceptibility checks, Williams, Cachaco, Gros Michel and Mbwazirume. The most stable genotypes were placed adjacent to the AEC abscissa and thus had the lowest projection onto the AEC ordinate. These genotypes were NARITA 2, 7, 14, 21, 18, 23, Cachaco and Gros Michel. Although NARITA 4, 6, 11 and 22 had a low overall disease severity, their response to black Sigatoka was less stable (Figure 3). On the other hand, Gros Michel, Cachaco and NARITA 18, although susceptible, were stable in their reaction to black Sigatoka across sites.

3.8. Discrimination Power and Representativeness of Test Environments

Based on the angles between the environment vectors, the four test sites were positively correlated in ranking of hybrid response to black Sigatoka (Figure 4).

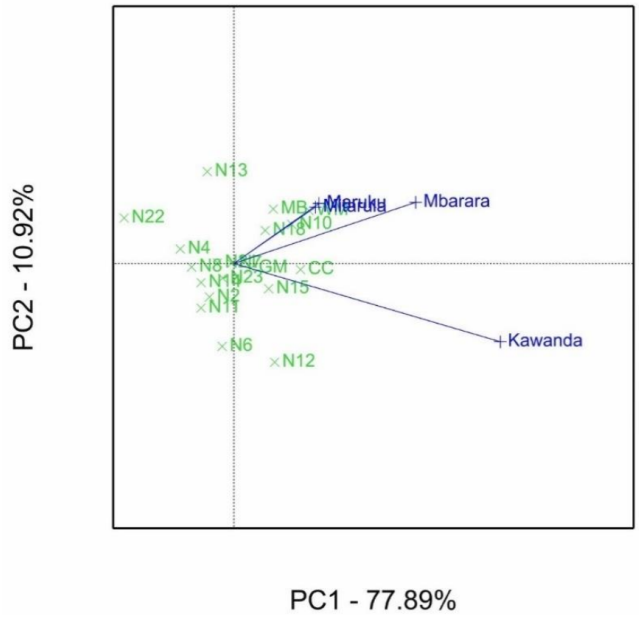

Figure 4. GGE biplot showing the relationship among the four test locations, based on black Sigatoka severity of 19 banana cultivars evaluated in Uganda and Tanzania.

Environment vectors for Maruku and Mitarula were almost equal in length with a very small acute angle between them, an indication that ranking of genotypes was most similar between the two locations. Mbarara had longer environment vectors than Maruku and Mitarula, indicating an enhanced ability to discriminate between hybrids, while Kawanda had the longest vector, and was thus the most discriminating (Figure 4).

Environments can be ranked based on their discriminativeness and representativeness of mega environments. The most ideal is located at the center of the concentric rings. The environment comparison biplot identified Mitarula as the most ideal test site for black Sigatoka screening, followed by Maruku (Figure 5).

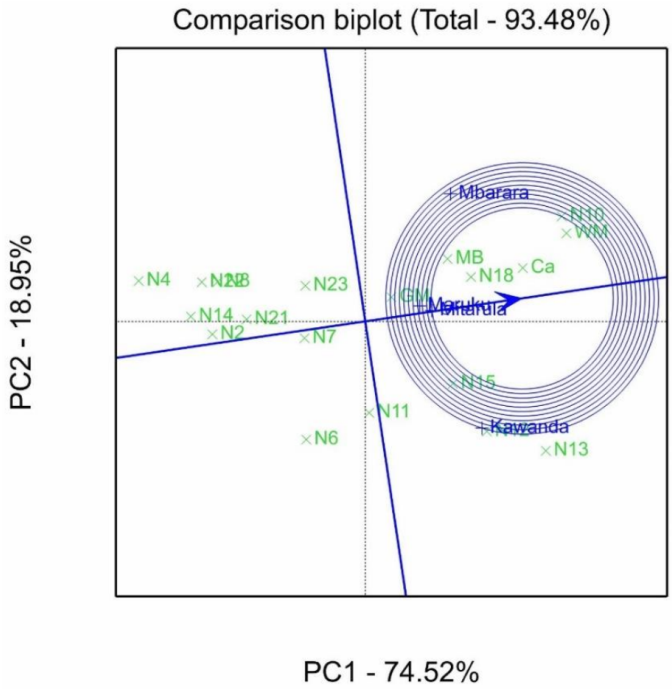

Figure 5. Discriminativeness and representativeness of the four testing sites based on the genotype x environment data of 19 banana cultivars (Matooke hybrids N—NARITA and banana varieties; MB—Mbwazirume, WM—Williams, Ca—Cachaco, GM—Gros Michel). The area of the inner circle of the biplot represents an ideal test environment, and the environment plotted within this circle is the best for Matooke hybrids and banana varieties evaluation. The location with the longest vector from the average environment axis and furthest from the ideal environment indicates high discriminativeness but poor representativeness.

3.9. Influence of Weather Variables on Disease Severity

There was no significant difference in the monthly average rainfall recorded between environments and plant cycles (Table 6). The highest mean monthly rainfall across disease cycles was at Mbarara (78.4 mm), and the lowest was at Maruku (57.2 mm).

For all environments, cycle 3 had the highest rainfall and cycle 1 had the lowest (Table 6). The locations and cycles also did not differ significantly in mean relative humidity. Mitarula had the highest RH (72.3%), and Mbarara had the lowest RH (67.7%). At Kawanda, cycle 2 had a higher RH (68.8%) than cycles 1 (68.4%) and 3 (68.7%), while cycle 1 had higher RH at Maruku (72.2%) and Mitarula (73.1%) than cycles 2 and 3. At Mbarara, cycle 3 had a higher RH (68.9%) than cycle 1 (67.3%) and 2 (67.2%) (Table 6). The lowest minimum temperature was recorded at Mitarula in cycle 1 (12.8 °C), while cycle 2 at Maruku had the highest minimum temperature (20.8 °C) (Table 6). The highest maximum temperature was recorded at Kawanda in cycle 3 (26.6 °C) and the lowest at Mitarula in cycle 1 (23.0 °C).

A regression model with AUDPC as the dependent variable, which combined RH, rainfall, minimum and maximum temperature, was highly significant ($p < 0.001$) and explained 45.7% of variation in disease severity among the regions (Table 7).

RH, maximum and minimum temperature significantly influenced disease severity, but rainfall had no significant effect on disease development. The regression equation indicated that maximum temperature was the most important factor influencing disease severity (Table 7).

Table 6. Environmental variables at four test sites in Uganda and Tanzania during the trial period 2016 to 2018.

	Monthly Rainfall (mm) *				Relative Humidity (RH %) *				Minimum Temperature (T_{min} °C) *				Maximum Temperature (T_{max} °C) *			
	Cycle 1	**Cycle 2**	**Cycle 3**	**Combined**	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Combined**	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Combined**	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Combined**
Kawanda	43.1 a	56.6 ab	88.0 b	60.6 ab	68.4 ab	68.8 ab	68.7 ab	68.2 ab	17.6 cd	18.4 d	18.6 de	18.1 d	26.2 cd	26.3 cd	26.6 cd	26.4 cd
Mbarara	65.3 ab	77.0 ab	97.2 b	78.4 ab	68.3 ab	67.2 ab	68.9 ab	67.7 ab	15.8 c	16.5 c	16.2 c	16.1 c	25.6 bc	26.0 cd	25.9 d	25.8 bc
Maruku	40.1 a	57.6 ab	79.6 ab	57.2 ab	72.2 b	66.0 a	67.3 ab	69.9 ab	19.2 de	20.8 f	20.5 f	20.1 f	23.2 ab	25.3 bc	25.3 b	24.5 b
Mitarula	53.8 ab	68.4 ab	81.4 ab	66.5 ab	73.1 b	71.1 ab	72.4 ab	72.9 b	12.8 a	13.5 a	13.9 ab	13.4 a	23.0 a	24.4 b	24.0 a	23.6 ab

* Means followed by the same letter in rows and columns for each parameter do not differ significantly at $p > 0.05$.

Table 7. Regression analysis of weather variables influencing black Sigatoka severity (AUDPC) at four test sites in Uganda and Tanzania.

Source	Coefficient Value	Standard Error	T (Standardized Coefficients)	*p* Value	Coefficient of Determination (R^2)
Intercept	−2676.347	730.904	−3.662	0.000	0.457
Rainfall	−0.038	0.024	−1.555	0.121	
RH	19.139	6.241	3.067	0.002	
T_{min}	−7.982	3.873	−2.061	0.040	
T_{max}	66.975	10.343	6.476	<0.0001	

RH—relative humidity, T_{min}—minimum temperature, T_{max}—maximum temperature.

4. Discussion

Managing banana diseases with host resistance requires the screening of plant material in multiple environments to identify hybrids with stable resistance and superior agronomical traits [43]. In this study, a set of NARITA hybrids were evaluated at two environments in Tanzania and two in Uganda. The test environments differed in disease pressure. In addition, the hybrids showed a range of responses from low disease severity to high susceptibility. Some hybrids responded differently to *P. fijiensis* infection in different environments. For example, NARITA 6 was ranked the best at Mbarara but not in other locations. This cultivar is a candidate for release in Mbarara. Five of the tested hybrids—NARITA 2, 7, 14, 21 and 23—had a similar response to *P. fijiensis* across sites. These hybrids with a stable response across environments, including NARITA 4, 11, 14 and 22, were less susceptible than Mbwazirume, the susceptibility check, and can be made available to smallholder banana farmers in the region as alternatives to black Sigatoka-susceptible Matooke cultivars if their agronomic traits and taste are acceptable to end users [44]. Following a preliminary evaluation in Uganda [30], NARITA 7 was selected and released to farmers in Uganda under the name KABANA 6H, code M9, locally nicknamed 'Kiwangaazi', meaning long-lasting [45]. In Tanzania, NARITA 4, 7 and 23 are recommended for release as alternatives to the highly susceptible local varieties.

In this study, NARITA 10, 12, 13, 15 and 18 were more susceptible to *P. fijiensis* than the local check Mbwazirume, contradicting findings of an earlier study conducted at Sendusu in Uganda [30], which reported all the 25 NARITA hybrids evaluated to have superior black Sigatoka resistance compared to Mbwazirume. The response of a cultivar to diseases depends on environmental conditions, the pathogen profile [33,46], and the host genotype. In this study, the environment was the greatest contributor (39.1%) to the variation in black Sigatoka severity. This study and earlier studies [33,47] reported a significant genotype x environment interaction. This interaction did not, however, lead to differential hybrid adaptation, as most of the hybrids were broadly adapted, as evidenced by consistent ranking of the hybrids across test sites. The exception is NARITA 6, which performed well at Mbarara only.

Of the weather variables recorded, RH and maximum temperature were strongly associated with black Sigatoka severity, concurring with earlier reports [48,49]. High RH is important in conidia formation. Conidia serves to initiate multiple reinfections during the growing season, increasing the number and size of lesions while maximum temperature influences the lesion formation, expansion and rate of leaf necrosis [50]. Rainfall appeared to have little or no impact on disease severity, contradictory to studies that reported that leaf wetness was critical for *P. fijiensis* spore germination, symptom development and disease severity [49,50]. Our observations suggest that conidia, whose germination is not affected by leaf wetness [50], is the main source of inoculum and played an important role in the spread and severity of black Sigatoka in this study.

Weather variables accounted for 45.7% of disease severity; thus, other environment specific factors such as soil nutrition levels and pathogen profiles may also have influenced Sigatoka severity. It has been reported that plantations rich in organic matter and soils high in silicon developed less disease [15,51–53]. There was, however, no direct relationship between organic matter content reported at each site and disease severity, and this could thus not explain differences in disease severity. The silicon levels were not measured, but this warrants further investigation as they could direct an integrated disease management strategy for black Sigatoka.

The presence of pathogen strains differing in virulence can result in different disease pressure between locations [33,46]. *Pseudocercospora fijiensis* is a heterothallic fungus that reproduces sexually. This mode of reproduction has the potential of creating new pathotypes differing in aggressiveness and virulence [54]. Both mating types of *P. fijiensis* were recovered among the isolates collected from all four sites, revealing the potential of sexual reproduction [55]. In addition, genetic characterization of *P. fijiensis* isolates collected from the test sites revealed extensive genetic diversity within and between sites [55]. It

is, therefore, possible that the differences in disease pressure between sites could have resulted from pathogens differing in virulence and aggressiveness. Characterization of *P. fijiensis* isolates from different sites is needed, to better understand pathogenic profiles.

Despite differences in the observed disease severity between locations, ranking of the hybrids was similar. This means that the same information on genotypes could be obtained from fewer locations, thus reducing the costs of disease evaluations. The clustering of evaluation sites into mega-environments identified Mitarula, Tanzania as the most discriminating and representative environment for black Sigatoka evaluation. Kawanda, where the Ugandan banana breeding program is based, showed a high discriminative ability but was not representative of all mega-environments. Although this site cannot be used to select the best black Sigatoka-resistant genotypes, it can still be used to discard highly susceptible genotypes [56].

The youngest leaf spotted and INSL are parameters widely used by breeders for assessing cultivar response to black Sigatoka [57–59]. These methods are, however, dependent on a plant's development stage, making the evaluation process expensive and delaying cultivar selection. AUDPC and mean DSI can therefore be used as alternative black Sigatoka assessment methods, as suggested by [57].

This study recommends Matooke hybrid selection for black Sigatoka at cycle 2 due to higher and consistent disease pressure. However, due to cost implications and to hasten the selection of materials for advancement, selection can be carried out at cycle 1 by establishing trials in known black Sigatoka hotspots such as Kawanda. To further increase disease pressure at cycle 1, highly susceptible cultivars should be established as spreaders prior to the trial establishment to act as inoculum sources [60]. In addition, leaves with Sigatoka symptoms can be spread within the trial to initiate disease epidemics [60].

In conclusion, this study identified seven NARITA hybrids with good levels of black Sigatoka resistance. These hybrids can be deployed for managing black Sigatoka in ECA. The final decision to deploy a variety should, however, be based on a combination of disease resistance, good agronomic traits such as yield and good culinary attributes for market and consumer acceptability [44]. Similarly, we identified the NARITA 10 hybrids as highly susceptible to black Sigatoka, and this can be used as a susceptibility check in future resistance evaluations. This study also confirmed the effectiveness of conventional breeding in developing banana hybrids resistant to *P. fijiensis*. The stability of released banana hybrids needs to be monitored over time as *P. fijiensis* reproduces sexually, which can result in a high genetic diversity of the fungus [55]. To save cost on resistance screening, we recommend a staged evaluation process where Kawanda in Uganda is used as a first testing site for discarding highly susceptible hybrids, and Mitarula, Tanzania, to identify disease-resistant hybrids for wider deployment. The pathogenicity of *P. fijiensis* isolates in ECA should be determined to select representative isolates that could be used for artificial inoculations.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11061145/s1, Table S1: Matooke hybrids (NARITAs) and banana varieties evaluated for response to black Sigatoka in Uganda and Tanzania during 2016-2018.

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