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Kathelyne Craenen · Rodomiro Ortiz

Effect of the bs_1 gene in plantain-banana hybrids on response to black sigatoka

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Abstract Use of resistant host genotypes is an important component of an integrated approach to control black sigatoka, a disease caused by the fungus *Mycosphaerella fijiensis* Morelet. The objective of the present research was to determine the role of the major gene for black sigatoka resistance (bs_1) in the host response to this disease. Euploid hybrids with a known genotype for the bs_1 locus were derived from triploid-diploid crosses of two French plantains and a diploid wild banana, and were assessed for their host response to black sigatoka in plant and ratoon crops in the humid forest zone of Nigeria. Host response was determined at flowering by recording the number of standing leaves, the youngest leaf with symptoms, the youngest leaf spotted, the total leaf area attacked by black sigatoka, and an index of the leaves spotted. An analysis of frequency distribution in each segregating population showed that almost all the traits displayed a normal distribution across ploidy level. This suggests that additive gene action plays an important role in the host-plant response to the fungus. Heritability, repeatibility, and intraclass correlations were calculated. The environment and the genotype-by-environment interaction significantly affected the host response to black sigatoka, which explains the low repeatibility of all traits. The intrafamily variation was larger than the interfamily variation, and most of the genetic variation in each family depended on the individual genotypes, regardless of their ploidy. The additive effect of, and the intralocus interaction at, the bs_1 locus on host response to black sigatoka were established by a one-way analysis of variance and regression analyses. Intralocus interaction in the bs_1 locus apparently regulates the appearance of symptoms on the leaf surface, whereas the additive effect and the intralocus interaction of the bs_1 locus affect disease development in the host plant. Therefore, the gene action(s) at the bs_1 locus may provide durable resistance to black sigatoka by slowing down disease development in the host plant.

Key words Durable resistance · Gene effects · *Musa* · *Mycosphaerella fijiensis* · Ploidy manipulations

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K. Craenen · R. Ortiz[1] (✉)
International Institute of Tropical Agriculture, c/o Lambourn and Co., Carolyn House, 26 Dingwall Road, Croydon, CR9 3EE, England, UK

Present address:
[1]Dept. of Agricultural Sciences, The Royal Veterinary and Agricultural University, 40, Thorvaldsensvej, DK 1871 Frederiksberg C, Copenhagen, Denmark

Introduction

Plantains and bananas (*Musa* spp. L.) rank among the ten most important food crops worldwide. They are grown in 120 countries in Africa, tropical America and Asia, where they contribute to the diet and the economic well-being of millions of people (INIBAP 1994). The major constraint to *Musa* production worldwide is a leaf spot disease called black sigatoka, caused by the fungus *Mycosphaerella fijiensis* Morelet. Leaf necrosis due to this disease reduces fruit yield by 30–50% in plantains (Stover 1983; Mobambo et al. 1993). Quarantine measures are not effective due to the dispersion of fungal spores by wind and water. Host-plant resistance is considered the most appropriate and sustainable component intervention for black sigatoka control (Vuylsteke et al. 1993 a). Host-plant resistance had an economic advantage over antifungal chemicals of 5.5:1 in normal periods and 10:1 in scarcity periods, given the price variations observed (Ortiz and Vuylsteke 1994 d).

Several plantain-banana tetraploid hybrids and plantain-derived diploids that are resistant to black

sigatoka have been developed by the International Institute of Tropical Agriculture (IITA) (Vuylsteke et al. 1993 b, 1995; Vuylsteke and Ortiz (1995). While tetraploid hybrids may be utilized directly as new cultivars by farmers in tropical countries, the plantain-derived diploids are a source of plantain alleles for germ plasm enhancement and genetic analysis at the diploid level (Ortiz 1995). Actually, few genetic studies were undertaken in this crop prior to 1992 (Simmonds 1952). Simmonds (1986) claimed that formal genetic studies of nearly or completely sterile triploids were impossible. As a consequence, very few genetic markers were available in *Musa* spp. until recently.

Several characteristics of the crop have made genetic analysis difficult. The low efficiency of generating hybrid progenies from interploidy/interspecific crosses and the resulting small sample sizes are major obstacles. However, it has been possible to obtain test-cross segregating populations by crossing triploid heterozygous plantain genotypes with the wild true-breeding banana, Calcutta 4 (Vuylsteke et al. 1993 c). The production of these types of segregating populations and their derived plantain euploid hybrids has now enabled the genetic analysis of plantain and banana genomes (Ortiz and Vuylsteke 1994 a, b, c, 1995 a, b; Ortiz et al. 1995 a, b; Vandenhout et al. 1995; Craenen and Ortiz 1996).

Resistance to black sigatoka in plantain-banana hybrids is conferred by a major recessive gene (bs_1) and two minor additive modifier genes (bsr_i), which enhance resistance to this disease (Ortiz and Vuylsteke 1994 a). Decreased stomatal density and increased pseudostem waxiness are two mechanisms of resistance, lengthening the incubation time of the disease in the leaves of resistant hybrids (K. Craenen and R. Ortiz, unpublished results). Pseudostem waxiness and stomatal density seem to be controlled by, or linked to, the modifier genes (bsr_i) in the host plant (Ortiz and Vuylsteke 1994 a; Ortiz et al. 1995 b). Hence, this experiment was performed to quantify the additive effect of the bs_1 allele, as well as the intralocus interaction in the bs_1 locus, on the host response to black sigatoka.

Materials and methods

A total of 114 euploid hybrids (Table 1), derived from crosses between triploid French plantains (*Musa* AAB group) Obino l' Ewai and Bobby Tannap, and the diploid AA wild banana (*M. acuminata* spp. *burmannicoides*) Calcutta 4 (Vuylsteke et al. 1993 c), were randomly planted in single-row plots of five plants at the IITA High Rainfall Station (southeastern Nigeria), where black sigatoka has a uniform widespread incidence, such that artificial inoculation was not required. Plants were spaced at 3×2 m (i.e. 30 m^2 for each experimental plot) and the experimental plots were surrounded by the susceptible False Horn plantain, cultivar Agbagba. Cultural practices, with the exception of fungicide treatment, were those recommended by Swennen (1990) for plantain cultivation in West Africa.

Table 1 Genotype of the bs_1 locus in euploid *Musa* hybrids evaluated for host plant response to black sigatoka

Ploidy	Genotype
Obino l'Ewai × Calcutta 4	
Diploid	11 heterozygous: 15 homozygous recessive
Triploid	2 simplex: 1 nulliplex
Tetraploid	8 duplex: 8 nulliplex
Bobby Tannap × Calcutta 4	
Diploid	25 heterozygous: 33 homozygous recessive
Triploid	2 simplex: 1 nulliplex
Tetraploid	6 duplex: 2 nulliplex

Genotyping of the bs_1 locus was confirmed by selfing or sib-mating a sample of euploid hybrids (Ortiz and Vuylsteke 1994 a). As expected, putative recessive or nulliplex genotypes for the bs_1 locus did not show segregation after selfing or sib-mating among themselves. However, the offspring of susceptible hybrids displayed all the phenotypic variation expected for heterozygous genotypes.

Host response to black sigatoka was assessed, once each at flowering in the plant crop and at flowering in the ratoon crop, from 1991 to 1993. The disease severity was measured as the youngest leaf spotted (YLS) (Vakili 1968), the youngest leaf with symptoms, the total leaf area with black sigatoka symptoms on a 0–6 scale (and also converted to percentage following Gauhl et al. 1995), and by an index of leaves with necrotic spots (Ortiz et al. 1993). The number of standing leaves (NSL) at flowering was also recorded. The index of leaves with necrotic spots (ILS) was calculated as follows: $ILS = 100 \times [NSL - (YLS - 1)]/NSL$. High values for the YLS and the youngest leaf with symptoms, and low records for total leaf area attacked by black sigatoka and the index of leaves with necrotic spots, indicate the presence of more healthy leaves, thereby implying a resistant host plant response to black sigatoka.

Data were analyzed as a nested analysis of variance (clone/ploidy/family) of the plant and ratoon crops (Ortiz 1995). The analysis took account of the unequal sample sizes among ploidies for each cross (Sokal and Rohlf 1981). Variance components were calculated based on plot means per cycle for genotypes (σ^2_G), environments (σ^2_E), genotype-by-environmental interaction (σ^2_{GE}/r), families (σ^2_F), ploidies within families ($\sigma^2_{X/P}$), and clones within ploidies within families ($\sigma^2_{C/X/P}$). This allowed the estimation of repeatability (R), broad-sense heritability (H^2) (Becker 1975) and intraclass correlation coefficients for families (r_F), ploidies (r_X), and clones (r_C) (Ortiz 1995) for host response to black sigatoka in the segregating euploid plantain-banana hybrids.

The normality of the frequency distribution of each trait for each family was analyzed using skewness (the degree of departure of a distribution from symmetry) and kurtosis (the gradient of the distribution as compared to that of a normal distribution), in order to infer the type of gene action for each trait involved in the host plant response to black sigatoka. The distribution homogeneity among euploids of the same ploidy and between ploidies was analyzed by a χ^2 test.

One-way analyses of variance were performed based on the genotype and the net effect of intralocus interaction of the bs_1 locus in euploid hybrids to determine their respective effects in the host plant response to black sigatoka. Linear correlations were calculated to establish independent associations between ploidy and the genetic parameters, with quantitative variation in host plant response to black sigatoka. Single and multiple linear regression analyses were carried out to determine the relationship between the genetic parameters (independent variables) and quantitative variation exhibited for black sigatoka by the euploid hybrids (dependent variable) across and within each family. Residual error values were tested by Durbin-Watson statistics to determine the reliability of the regression models (Sokal and Rohlf 1981).

Results and discussion

There was a significant interaction between genotype and production cycle (or environment) for most of the methods used to assess host plant response to black sigatoka. Also the environment (or production cycle) significantly affected the youngest leaf with symptoms, the youngest leaf spotted, and the total leaf area attacked by black sigatoka (in percentage). However, the analyses of variances (Table 2) suggested that genotypic differences accounted for most of the phenotype variation in host response to this disease. Significant differences were observed between genotypes within each ploidy ($P < 0.05$ or $P < 0.001$) in diploid progeny from both crosses and in tetraploid progeny from Obino l' Ewai × Calcutta 4. Means for host response to black sigatoka at flowering across cycles, families, and ploidies within families, are shown in Table 3.

As shown by the analyses of variance (Table 2) most of the genetic variation (measured by the H^2) depended

Table 2 Analyses of variance for host response to black sigatoka at flowering, as measured by the number of standing leaves (NSL), the youngest leaf spotted (YLS), the youngest leaf with symptoms (YLWS), the total leaf area attacked by black sigatoka on a 0–6 scale (BSLA) or on percentage scale (TLABS), and the index of leaves with necrotic spots (ILS %), in euploid hybrids derived from crosses between the French plantains Obino l' Ewai (OL) and Bobby Tannap (BT) with the wild banana Calcutta 4 (C4). (Onne, Nigeria, 1991–1993, plant and ratoon crops)

Source of variation	Degrees of freedom	Mean squares and significance of *F*-tests					
		NSL	YLS	YLWS	BSLA	TLABS (%)	ILS (%)
Cycle (C)	1	114.90*	27.98***	65.57***	0.02 NS	152.34*	144.32 NS
Genotypes (G)	113	5.16*	4.40***	4.36***	0.26***	59.20***	140.73***
Family (F)	1	4.93 NS	18.27 NS	2.28 NS	0.86 NS	265.42 NS	533.54 NS
Ploidy (X)/F	4	4.76 NS	9.43 NS	4.63 NS	0.31 NS	42.33 NS	290.83 NS
Ploidy/OL × C4	2	6.57 NS	14.15 NS	5.68 NS	0.29 NS	24.66 NS	394.16 NS
Ploidy/BT × C4	2	2.95 NS	4.71 NS	3.58 NS	0.32 NS	59.81 NS	187.50 NS
Clones/X/F	108	5.19***	4.08***	4.38***	0.25**	57.06**	129.67*
2*x*/OL × C4	25	4.32**	2.49 NS	3.28*	0.20*	41.38*	88.55 NS
3*x*/OL × C4	2	4.21 NS	2.17 NS	1.98 NS	0.04 NS	32.25 NS	16.61 NS
4*x*/OL × C4	15	6.30**	5.46**	3.58***	0.15 NS	42.76 NS	83.23 NS
2*x*/BT × C4	57	5.56***	4.72***	5.56***	0.32 NS	70.96*	165.51 NS
3*x*/BT × C4	2	4.45 NS	0.20 NS	0.17 NS	0.34 NS	115.01 NS	156.64 NS
4*x*/BT × C4	7	3.41 NS	3.21 NS	2.35 NS	0.14 NS	26.52 NS	108.62 NS
G × C interaction	85	1.84**	1.88**	1.86***	0.14**	32.97 NS	88.64*
Coefficient of variation (%)		13.1	17.2	19.5	29.8	48.5	30.3

*, **, *** indicate significance at the 5%, 1%, and 0.1% levels, respectively

Table 3 Host response by cycle and ploidy within family to black sigatoka at flowering, as measured by the number of standing leaves (NSL), the youngest leaf spotted (YLS), the youngest leaf with symptoms (YLWS), the total leaf area attacked by black sigatoka on a 0–6 scale (BSLA) or on percentage scale (TLABS), and the index of leaves with necrotic spots (ILS), in euploid hybrids derived from crosses between the French plantains Obino l' Ewai (OL) and Bobby Tannap (BT) with wild banana Calcutta 4 (C4). (Onne, Nigeria, 1991–1993). See Table 2 for tests of significance

Item	NSL	YLS	YLWS	BSLA	TLABS (%)	ILS (%)
Cycles						
Plant crop	10.9	8.2	7.4	1.3	11.2	33.1
Ratoon crop	9.7	7.7	6.5	1.3	12.7	30.9
Ploidy within Family						
Obino l'Ewai × Calcutta 4	10.6	8.4	7.1	1.2	10.4	30.1
Diploids	10.5	8.3	7.3	1.2	10.7	29.9
Triploids	9.2	6.3	5.8	1.5	12.5	42.0
Tetraploids	10.9	8.8	7.1	1.1	9.6	28.4
Bobby Tannap × Calcutta 4	10.3	7.7	6.9	1.3	12.7	33.4
Diploids	10.3	7.7	6.8	1.3	12.9	33.9
Triploids	9.2	6.8	6.0	1.5	15.7	35.4
Tetraploids	10.4	8.3	7.4	1.1	10.4	28.6

Table 4 Variance components, based on plot means per cycle, of genotypes (σ^2_G), environments (σ^2_E), genotype-by-environment interaction (σ^2_{GE}/r), families (σ^2_F), ploidies within families ($\sigma^2_{X/P}$), and clones within ploidies within familes ($\sigma^2_{C/X/P}$), repeatability (R), broad-sense heritability (H^2), intraclass correlation coefficients for families (r_F), ploidies (r_X), and clones (r_C), for host response to black sigatoka at flowering, as measured by the number of standing leaves (NSL), the youngest leaf spotted (YLS), the youngest leaf with symptoms (YLWS), the total leaf area attacked by black sigatoka on a 0–6 scale (BSLA) or on a percentage scale (TLABS), and the index of leaves with necrotic spots (ILS), in segregating euploid plantain-banana hybrids (Onne, Nigeria, 1991–1993, plant and ratoon crops)

Variance component genetic parameter	NSL	YLS	YLWS	BSLA	TLABS (%)	ILS (%)
σ^2_G	1.66***	1.26***	1.25***	0.06***	13.12***	26.04**
σ^2_E	1.15***	0.26***	0.65***	$\simeq$ 0.00	1.21*	0.57
σ^2_{GE}/r	0.92**	0.94**	0.93***	0.08**	16.48	44.32*
σ^2_F	$\cong$ 0.00	$\cong$ 0.00	$\cong$ 0.00	$\cong$ 0.00	$\cong$ 0.00	$\cong$ 0.00
$\sigma^2_{X/P}$	$\cong$ 0.00	$\cong$ 0.00	$\cong$ 0.00	$\cong$ 0.00	$\cong$ 0.00	$\cong$ 0.00
$\sigma^2_{C/X/P}$	3.22***	2.12***	2.42***	0.10**	23.12**	39.37*
$R = \sigma^2_G/[\sigma^2_E + \sigma^2_{GE}]$	0.56	0.59	0.49	0.33	0.38	0.29
$H^2 = \sigma^2_G/[\sigma^2_E + \sigma^2_{GE}/r]$	0.64	0.57	0.57	0.45	0.44	0.37
$r_F = \sigma^2_F/[\sigma^2_F + \sigma^2_{X/F} + \sigma^2_{C/X/F}]$	$\cong$ 0.00	$\cong$ 0.00	$\cong$ 0.00	$\cong$ 0.00	$\cong$ 0.00	$\cong$ 0.00
$r_X = \sigma^2_{X/F}/[\sigma^2_{X/F} + \sigma^2_{C/X/F}]$	$\cong$ 0.00	$\cong$ 0.00	$\cong$ 0.00	$\cong$ 0.00	$\cong$ 0.00	$\cong$ 0.00
$r_C = \sigma^2_{C/X/P}/[\sigma^2_{C/X/P} + \sigma^2_{GE}/r]$	0.78	0.69	0.72	0.58	0.58	0.47

*, **, *** indicate significance at the 5% and 1%, and 0.1% levels, respectively

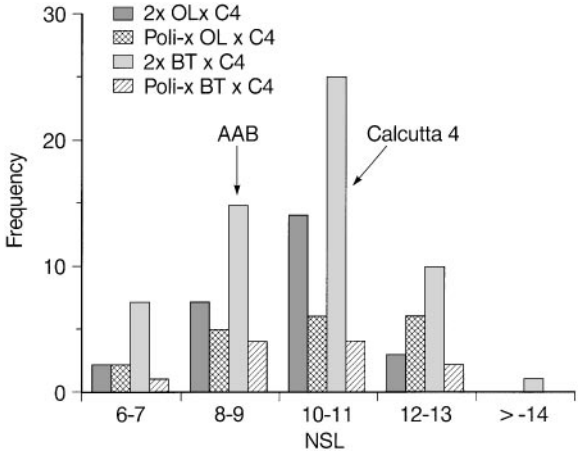

Fig. 1 Frequency distribution for the number of standing leaves (*NSL*) at flowering in euploid plantain-banana hybrids. *Arrows* indicate values for plantains (*AAB*) and Calcutta 4

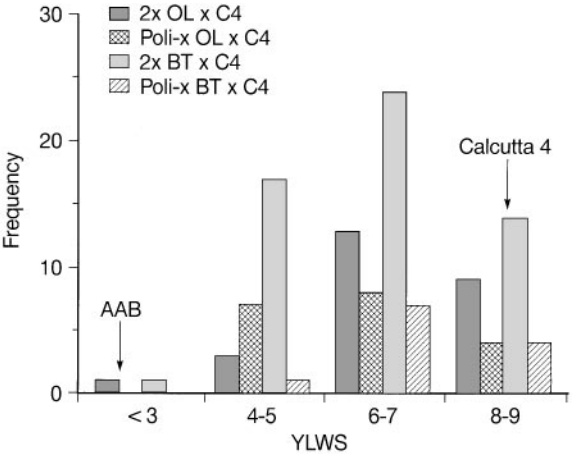

Fig. 2 Frequency distribution for the youngest leaf with symptoms (*YLWS*) at flowering in euploid plantain-banana hybrids. *Arrows* indicate values for plantains (*AAB*) and Calcutta 4

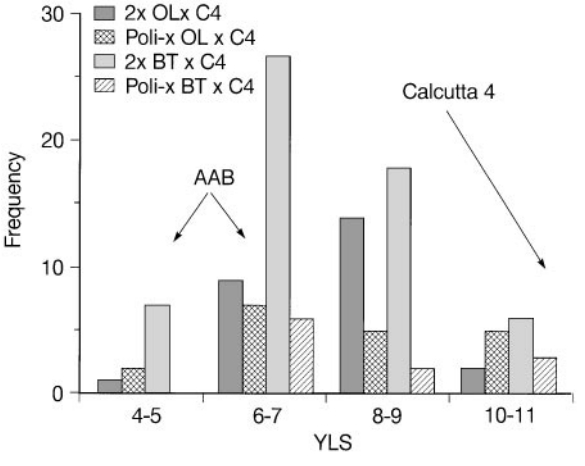

Fig. 3 Frequency distribution for the youngest leaf spotted (*YLS*) at flowering in euploid plantain-banana hybrids. *Arrow* indicate values for plantains (*AAB*) and Calcutta 4 (out of scale, i.e. without necrotic spots)

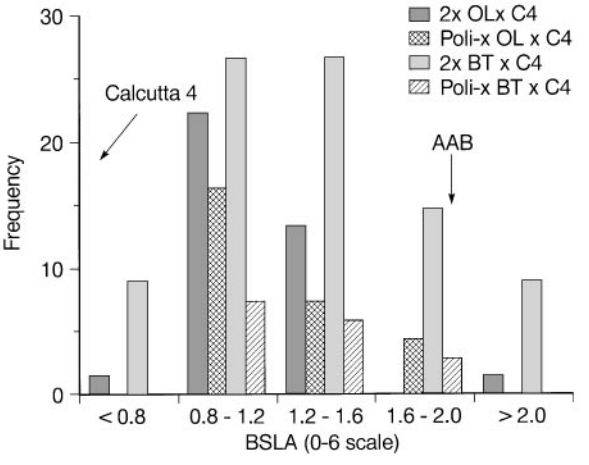

Fig. 4 Frequency distribution for the total leaf area attacked by black sigatoka on a percentage scale (*TLABS*) at flowering in euploid plantain-banana hybrids. *Arrows* indicate values for plantains (*AAB*) and Calcutta 4 (out of scale)

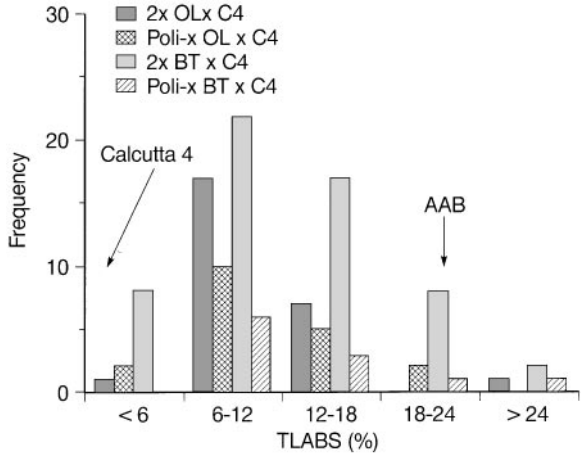

Fig. 5 Frequency distribution for the total leaf area attacked by black sigatoka on a percentage scale (*TLABS*) at flowering in euploid plantain-banana hybrids. *Arrows* indicate values for plantains (*AAB*) and Calcutta 4

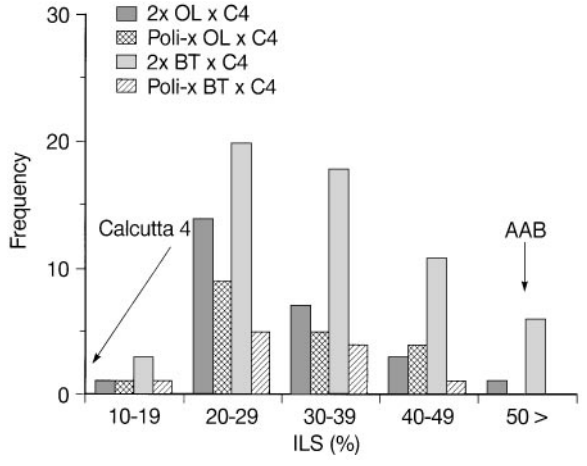

Fig. 6 Frequency distribution for the index of leaf spotted at flowering (*ILS*) at flowering in euploid plantain-banana hybrids. *Arrows* indicate values for plantains (*AAB*) and Calcutta 4 (out of scale, i.e. 0)

on the individual genotypes, irrespective of their ploidy in each family (Table 4). This explained the high values of r_C, i.e., the intrafamily variability was larger than the interfamily variability. Hence, as suggested by Ortiz (1995), individual selection within each family should be most appropriate to develop black sigatoka-resistant germ plasm. The repeatibility values (R) were low (Table 4) because both the environment and the genotype-by-environment interaction significantly affected host response (Table 2). It seems that the plants showed more susceptibility in the ratoon than in the plant crop (Table 3). Hence, selection of resistant genotypes should be made in the ratoon crop because plantain and banana are perennial crops.

Table 5 Statistical and genetic analysis of frequency distribution of host response to black sigatoka in euploid hybrids derived from crosses between the French plantains Obino l' Ewai (OL) and Bobby Tannap (BT) with the wild banana Calcutta 4 (C4). (Onne, Nigeria, 1991–1993)

Number of standing leaves	
χ^2 (homogeneity)	
Among diploids	1.60; $P = 0.81$
Among polyploids	1.42; $P = 0.70$
Between ploidies	2.87; $P = 0.58$
Skewness and kurtosis were non-significant ($P > 0.05$) for all frequency distributions. Transgressive segregation was observed due to epistasis	
Youngest leaf spotted	
χ^2 (homogeneity)	
Among diploids	4.46; $P = 0.22$
Among polyploids	1.86; $P = 0.60$
Between ploidies	6.24; $P = 0.10$
Skewness and kurtosis were non-significant ($P > 0.05$) for all frequency distributions. The sample size does not enable one to recover the C4-host plant response in segregating offspring	
Youngest leaf with symptoms	
χ^2 (homogeneity)	
Among diploids	3.67; $P = 0.30$
Among polyploids	3.15; $P = 0.21$
Between ploidies	0.87; $P = 0.83$
Skewness and kurtosis were non-significant ($P > 0.05$) for all frequency distributions except in polyploids of BT × C4 ($P < 0.05$ for skewness and $P < 0.01$ for kurtosis). Additive gene action of few genes since both parental phenotypes were recovered	
Total leaf area attacked by black sigatoka (0–6 scale)	
χ^2 (homogeneity)	
Among diploids	9.62; $P = 0.05$*
Among polyploids	0.46; $P = 0.79$
Between ploidies	6.42; $P = 0.17$
Skewness and kurtosis were non-significant ($P > 0.05$) for all frequency distributions except in polyploids of OL × C4 ($P < 0.05$). Sample size does not enable one to recover the C4-host plant response in segregating offspring	
Total leaf area attacked by black sigatoka (in a percentage scale)	
χ^2 (homogeneity)	
Among diploids	8.14; $P = 0.09$
Among polyploids	2.91; $P = 0.57$
Between ploidies	0.62; $P = 0.96$
Skewness and kurtosis were non-significant ($P > 0.05$) for all frequency distributions. Sample size does not enable one to recover the C4-host plant response in segregating offspring	
Index of leaves with necrotic spots (%)	
χ^2 (homogeneity)	
Among diploids	3.33; $P = 0.50$
Among polyploids	0.99; $P = 2.80$
Between ploidies	2.85; $P = 0.58$
Skewness and kurtosis were non-significant ($P > 0.05$) for all frequency distributions. Sample size does not enable one to recover the C4-host plant response in segregating offspring	

The analyses of frequency distribution for the number of standing leaves at flowering (Fig. 1), the host response to black sigatoka at flowering (as measured by the youngest leaf spotted: Fig. 2), the youngest leaf with

Table 6 Analyses of variance of host response to black sigatoka at flowering, as measured by the number of standing leaves (NSL), the youngest leaf spotted (YLS), the youngest leaf with symptoms (YLWS), the total leaf area attacked by black sigatoka on a 0–6 scale (BSLA) or on a percentage scale (TLABS), and the index of leaves with necrotic spots (ILS), based on the genotype and net effect of intralocus interaction of the bs_1 locus in euploid hybrids derived from crosses between the French plantains Obino l' Ewai and Bobby Tannap with the wild banana Calcutta 4. (Onne, Nigeria, 1991–1993)

Source of variation	Degrees of freedom	Mean squares and significance of *F*-tests					
		NSL	YLS	YLWS	BSLA	TLABS (%)	ILS (%)
Genotype bs_1 locus							
Environment (E)	1	80.37***	22.17***	43.20***	0.03	156.54	176.21
Frequency allele (bs_1)	3	45.35***	51.78***	22.26***	1.55***	385.50***	811.89***
E × bs_1 interaction	3	2.48	3.08	1.34	0.04	39.10	21.40
Coefficient of variation (%)		16.8	21.0	24.6	34.5	55.3	32.3
Net intralocus effect in bs_1							
Environment (E)	1	83.91***	23.26***	44.95***	0.03	157.71	179.24
Intralocus effect (I)	4	49.61***	49.06***	27.19***	1.11***	281.18***	580.97***
E × I interaction	4	6.73*	4.05	4.91	0.24	76.70	86.42
Coefficient of variation		15.8	20.2	23.7	34.4	55.0	32.4

*, **, indicate significance at the 5% and 1% levels, respectively

Table 7 Adjusted least square means, based on the genotype of and the net effect of intralocus interaction at the bs_1 locus, for host response to black sigatoka at flowering, as measured by the number of standing leaves (NSL), the youngest leaf spotted (YLS), the youngest leaf with symptoms (YLWS), the total leaf area attacked by black sigatoka on a 0–6 scale (BSLA) or on a percentage scale (TLABS), and the index of leaves with necrotic spots (ILS), in euploid hybrids derived from crosses between the French plantains Obino l' Ewai and Boby Tannap with the wild banana Calcutta 4. (Onne 1991–1993). *n* = sample size (number of clones in brackets)

Item	*n*	NSL	YLS	YLWS	BSLA	TLABS (%)	ILS (%)
Frequency of bs_1 allele							
4	19 (10)	12.2	10.1	7.9	1.0	6.8	24.7
3	4 (2)	10.6	7.3	6.6	1.7	17.6	40.6
2	120 (66)	10.6	8.2	7.2	1.2	11.1	31.1
1	57 (36)	9.3	7.0	6.2	1.5	14.6	34.2
$LSD_{0.05}/[1/N_1 + 1/N_2]^{1/2}$		3.4	3.3	3.4	0.9	12.9	20.5
Net intralocus interaction at the bs_1 locus							
4 = nulliplex, tetraploid	19 (10)	12.1	10.1	7.9	1.0	6.7	24.7
3 = nulliplex, triploid	4 (2)	10.6	7.3	6.7	1.7	17.4	40.7
2 = homozygous recessive, diploid	89 (48)	11.0	8.6	7.6	1.2	10.6	30.6
1 = simplex, triploid	7 (4)	8.4	6.2	5.4	1.4	12.4	37.1
0 = heterozygous diploid or duplex tetraploid	81 (50)	9.4	7.1	6.3	1.4	14.0	34.8
$LSD_{0.05}/[1/N_1 + 1/N_2]^{1/2}$		3.2	3.2	3.3	0.9	12.8	20.5

symptoms (Fig. 3), the total leaf area attacked by black sigatoka disease on a 0 to 6 scale (Fig. 4) or on a percentage scale (Fig. 5), and the index of leaves with necrotic spots (Fig. 6) are shown in Table 5. Almost all the traits showed a normal distribution, irrespective of their ploidy level. Hence, additive gene action may play an important role in the host response to black sigatoka. The euploid hybrids had their number of standing leaves at flowering beyond the limits of variation defined by the parents (Fig. 1). This observed transgressive segregation may be due to epistasis. Due to the small sample size, no hybrids were observed to have the 'highly resistant' phenotype of Calcutta 4. This suggests that the resistance to black sigatoka of Calcutta 4 could have a polygenic basis.

All genetic analyses (Tables 4 and 5) suggest that both Obino l' Ewai and Bobby Tannap have similar genotypes for the bs_1 locus, as reported earlier by Ortiz and Vuylsteke (1994 a). Analyses of variance of host response to black sigatoka, based on the genotype and net effect of intralocus interaction of the bs_1 locus are shown in Table 6. Genotype-by-environment interaction was not important when the analyses were based on the marker genotype. This was expected since Mendelian genetic markers are seldom affected by the environment.

The host resistance to black sigatoka was significantly enhanced by an increase in the bs_1 allele frequency and the interactions at the bs_1 locus, as shown by the least square means (Table 7). The means for

Table 8 Simple regression and correlation anayses based on genetic effects for host response to black sigatoka at flowering, as measured by the number of standing leaves (NSL), the youngest leaf spotted (YLS), the youngest leaf with symptoms (YLWS), the total leaf area attacked by black sigatoka on a 0–6 scale (BSLA) or on percentage scale (TLABS), and the index of leaves with necrotic spots (ILS), in euploid hybrids derived from crosses between the French plantains Obino l' Ewai (OL) and Bobby Tannap (BT) with wild banana Calcutta 4 (C4). (Onne, Nigeria, 1991–1993). n = number of clones

Analysis/family	n	TNL	YLS	YLWS	BSLA	TLABS (%)	ILS (%)
Correlation coefficients between bs_1 intralocus interaction and host response to black sigatoka disease							
Combined	114	0.53***	0.58***	0.43***	− 0.34***	− 0.34***	− 0.36***
OL × C4	45	0.72***	0.70***	0.52**	− 0.38***	− 0.44**	− 0.34**
BT × C4	69	0.34***	0.47***	0.37***	− 0.30*	− 0.25*	− 0.36**
Regression models between bs_1 intralocus interaction (X_1) and host response to black sigatoka (Y)							
Combined	114	$9.4 + 0.7X_1$	$6.9 + 0.7X_1$	$6.2 + 0.5X_1$	$1.4 - 0.1X_1$	$14.3 - 1.7X_1$	$36.9 - 2.7X_1$
OL × C4	45	$9.2 + 0.8X_1$	$7.8 + 0.7X_1$	$6.2 + 0.5X_1$	$1.4 - 0.4X_1$	$13.5 - 1.7X_1$	$33.4 - 1.8X_1$
BT × C4	69	$9.5 + 0.6X_1$	$6.9 + 0.7X_1$	$6.2 + 0.5X_1$	$1.5 - 0.1X_1$	$14.6 - 1.5X_1$	$37.8 - 3.3X_1$
Correlation coefficient between bs_1 allele frequency and host response to black sigatoka disease							
Combined	114	0.44***	0.54***	0.35***	− 0.32***	− 0.32***	− 0.36***
OL × C4	45	0.59***	0.60***	0.35*	− 0.31*	− 0.37*	− 0.27
BT × C4	69	0.32**	0.48***	0.38***	− 0.33**	− 0.26*	− 0.42**
Regression models between bs_1 allele frequency (X_2) and host response to black sigatoka (Y)							
Combined	114	$8.5 + 0.9X_2$	$5.9 + 1.1X_2$	$5.6 + 0.7X_2$	$1.6 - 0.2X_2$	$16.8 - 2.5X_2$	$40.6 - 4.2X_2$
OL × C4	45	$8.2 + 1.0X_2$	$6.1 + 1.0X_2$	$5.8 + 0.5X_2$	$1.5 - 0.1X_2$	$15.6 - 2.2X_2$	$35.5 - 2.3X_2$
BT × C4	69	$8.7 + 0.8X_2$	$5.6 + 1.2X_2$	$5.2 + 1.0X_2$	$1.7 - 0.2X_2$	$17.4 - 2.7X_2$	$45.2 - 6.6X_2$

*, **, *** indicate significance at the 5%, 1% and 0.1% levels, respectively

triploids should be approached with caution due to their small sample size, improper assignment of genotypes, or imbalance of gene action due to triploidy. Nonetheless, the disease developed more slowly in the partially resistant triploids than in their full-sib susceptible triploids, but not significantly so.

Simple regression and correlation analyses revealed that genetic effects ($P < 0.05$) rather than ploidy level $P > 0.05$) affected the host response to black sigatoka. However, a sgnificant dosage effect (Stern 1929) may be expected since there was a significant correlation between the bs_1 allele frequency and host response to black sigatoka (Table 8). The correlations between ploidy levels and the descriptors for black sigatoka host response were not significant ($P > 0.05$) either within or between crosses.

Multiple regression models based on intralocus interactions (X_1) and allele frequency (X_2) at the bs_1 locus (Table 9) were significant for the following descriptors (Y_1) scored at flowering: the number of standing leaves, the youngest leaf spotted, the youngest leaf with symptoms, the total leaf area with black sigatoka symptoms on a 0–6 scale or on a percentage scale (except in Bobby Tannap × Calcutta 4), and the index of leaves with necrotic spots (except in Obino l' Ewai × Calcutta 4). The net effect of the intralocus interaction at the bs_1 locus was significantly more important than the additive effect (as measured by the bs_1 allele frequency) for the total number of leaves and the youngest leaf with symptoms (YLWS). However, both were equally important for the youngest leaf spotted when results were averaged across the two crosses. This was determined by the significance of the partial regression coefficients (Table 9). Non-significant regression coefficients should be discarded from the statistical model because they were initially considered due to their significant independent correlation with the other independent variable.

This analysis suggests that intralocus interactions (i.e. the net effect of the bs_1 locus) controlled the appearance of black sigatoka symptoms on the leaf surface. However, both the additive effect of, and the intralocus interaction at, the bs_1 locus controlled the development of the disease in the host plant, as measured by the youngest leaf spotted. The value for the youngest leaf spotted indicates the presence of fully functional leaves on the plant. Less leaf spot damage ensues from slow or delayed disease symptoms (Vuylsteke and Ortiz 1995).

Hence, the overall gene action of the bs_1 locus may provide durable resistance to black sigatoka because it slows disease development in the host plant. As a consequence, resistant hybrids show more healthy leaves, i.e., more photosynthetic leaf area, than their susceptible full-sibs. This may partially account for their high yields. Results from multilocational trials in Africa showed that all partially resistant hybrids had a homeostatic resistant host response to black sigatoka (Ortiz et al. 1995 c). Also, some of them achieved high and stable yields across environments due to their black sigatoka resistance (Ortiz and Vuylsteke 1995 c).

This study implies that disease onset, as measured by the youngest leaf spotted and youngest leaf with

Table 9 Multiple regression models based on net effect of intralocus interactions (X_1) and allele frequency (X_2) in the bs_1 locus for host response to black sigatoka at flowering, as measured by the number of standing leaves (NSL), the youngest leaf spotted (YLS), the youngest leaf with symptoms (YLWS), the total leaf area attacked by black sigatoka on a 0–6 scale (BSLA) or on a percentage scale (TLABS), and the index of leaves with necrotic spots (ILS), in euploid hybrids derived from crosses between the French plantains Obino l' Ewai (OL) and Bobby Tannap (BT) with the wild banana Calcutta 4 (C4). (Onne, Nigeria, 1991–1993). Multiple regression model ± standard error

Model ± standard error	R^2 (%)	*F*-test	*P*	Durbin-Watson statistics (d)[a]
Combined over two families ($n = 114$)				$d_L = 1.63$; $d_U = 1.72$
$TNL = 9.5 + 0.8^{**}X_1 - 0.1X_2 \pm 1.5$	27.9	21.47	$< 0.001^{***}$	2.063
$YLS = 10.4 - 0.1^{**}X_1 + 0.6^{**}X_2 \pm 1.0$	66.5	110.23	$< 0.001^{***}$	2.019
$YLWS = 6.4 + 0.6^{**}X_1 - 0.2X_2 \pm 1.4$	18.4	12.48	$< 0.001^{***}$	1.886
$BSLA = 1.5 - 0.1X_1 - 0.1X_2 \pm 0.4$	11.7	7.35	0.001^{***}	1.680
$TLABS = 15.3 + 1.2X_1 - 0.9X_2 \pm 6.0$	11.9	7.51	$< 0.001^{***}$	1.648
$ISL = 38.9 + 1.3X_1 - 2.5X_2 \pm 9.0$	13.8	9.00	$< 0.001^{***}$	1.908
OL × C4 ($n = 45$)				$d_L = 1.46$; $d_U = 1.63$
$TNL = 9.7 + 1.1^{**}X_1 - 0.4X_2 \pm 1.2$	53.0	23.66	$< 0.001^{***}$	1.752
$YLS = 7.3 - 0.8^{**}X_1 - 0.2X_2 \pm 1.2$	49.1	20.26	$< 0.001^{***}$	1.435
$YLWS = 7.2 + 1.0^{**}X_1 - 0.8X_2 \pm 1.2$	32.7	10.22	$< 0.001^{***}$	1.679
$BSLA = 1.3 - 0.1X_1 + 0.04X_2 \pm 0.4$	14.5	3.57	0.037^{*}	1.292
$TLABS = 12.7 - 2.1X_1 + 0.6X_2 \pm 5.4$	19.8	5.18	0.001^{***}	1.242
$ISL = 32.2 - 2.4X_1 + 0.9X_2 \pm 8.0$	11.5	2.73	0.077	1.722
BT × C4 ($n = 69$)				$d_L = 1.55$; $d_U = 1.67$
$TNL = 9.5 + 0.5X_1 + 0.05X_2 \pm 1.6$	13.4	5.10	0.009^{**}	2.117
$YLS = 6.1 + 0.3X_1 + 0.7X_2 \pm 1.4$	24.0	10.43	$< 0.001^{***}$	2.109
$YLWS = 5.5 + 0.2X_1 + 0.6X_2 \pm 1.6$	15.1	5.86	0.005^{**}	2.038
$BSLA = 1.7 - 0.03X_1 - 0.2X_2 \pm 0.4$	10.7	3.97	0.023^{*}	1.688
$TLABS = 16.5 - 0.6X_1 - 1.7X_2 \pm 6.5$	7.1	2.54	0.087	1.633
$ISL = 44.8 - 0.3X_1 - 6.1X_2 \pm 9.4$	17.3	6.88	0.002^{**}	2.019

*, **, *** indicate significance at the 5%, 1% and 0.1% levels, respectively

[a] If $d_U < d < 4 - d_U$ the hypothesis (H_0) that $\rho = 0$ (no serial correlation) is not rejected; if $d < d_L$ or $d > 4 - d_L$ the H_0 is rejected, hence, serially correlated data because $\rho <> 0$; if $d_L < d < d_U$ or $4 - d_U < d < 4 - d_L$, the test is inconclusive

symptoms, is an indication of a specific gene for gene response. This was clearly shown in all the analyses. However, it was not clear from our research what other factors could have affected black sigatoka disease development in *Musa*, as measured by the number of standing leaves, the leaf area attacked by black sigatoka, and the index of leaves with necrotic spots. An epidemiological study, which considers other factors involved in host plant-pathogen-environment interactions (as suggested by Thal et al. 1992), may provide the needed insights.

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