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Inheritance of black sigatoka disease resistance in plantain-banana (*Musa* spp.) hybrids

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Abstract Black sigatoka (*Mycosphaerella fijiensis* Morelet), an airborne fungal leaf-spot disease, is a major constraint to plantain and banana (*Musa* spp.) production world-wide. Gaining further knowledge of the genetics of host-plant resistance will enhance the development of resistant cultivars, which is considered to be the most appropriate means to achieve stable production. Genetic analysis was conducted on 101 euploid (2*x*, 3*x* and 4*x*) progenies, obtained from crossing two susceptible triploid plantain cultivars with the resistant wild diploid banana 'Calcutta 4'. Segregating progenies, and a susceptible reference plantain cultivar, were evaluated over 2 consecutive years. Three distinct levels of host response to black sigatoka were defined as follows: susceptible (< 8 leaves without spots), less susceptible (8–10) and partially resistant (> 10). Segregation ratios for resistance at the 2*x* level fitted a genetic model having one major recessive resistance allele (bs_1) and two independent alleles with additive effects (bsr_2 and bsr_3). A similar model explains the results at the 4*x* level assuming that the favourable resistance alleles have a dosage effect when four copies of them are present in their respective loci (bs_i^4). The proposed model was further validated by segregation data of S_1 progenies. Mechanisms of black sigatoka resistance are discussed in relation to the genetic model.

Key words 2n eggs · Horizontal durable resistance · *Mycosphaerella fijiensis* · Resistance mechanisms · Trisomic segregation

Introduction

Plantains (*Musa* spp., AAB group) are triploid (2n = 3x = 33) giant perennial herbs grown throughout the tropics. They have evolved from interspecific crosses between the diploid (2n = 2x = 22) species *M. acuminata* Colla and *M. balbisiana* Colla, which contributed the A and B genomes, respectively (Stover and Simmonds 1987). Black sigatoka (BS), the airborne fungal leaf-spot disease caused by *Mycosphaerella fijiensis* Morelet, is a major constraint to plantain and banana production (Stover and Simmonds 1987). Resistance breeding is generally considered the most appropriate technology to control BS leaf spot. Therefore, in 1987, the International Institute of Tropical Agriculture (IITA) initiated a genetic improvement program targeting the incorporation of durable host-plant resistance to black sigatoka in plantain and banana (Vuylsteke et al. 1993a). Highly-resistant diploid bananas were identified and used in the breeding program. Since then, several hundred euploid (2x, 3x and 4x) plantain-banana hybrids with black sigatoka resistance have been obtained via 3x × 2x crosses (Vuylsteke et al. 1993b).

The genetics and inheritance of black sigatoka resistance has not yet been elucidated. However, the production of diploids from 3x × 2x crosses and the occurrence of a second-division restitution (SDR) mechanism in the production of 2n eggs by the plantain parent (Vuylsteke et al. 1993b) offers opportunity for both genetic analysis and improvement of *Musa* spp. This paper provides insight into the genetics of black sigatoka resistance (BSR) in plantain and banana. It also discusses the proposed genetic model in relation to potential mechanisms of BSR that have been reported (Mourichon et al. 1990; Sallé et al. 1990; Vazquez et al. 1990).

Materials and methods

Two triploid plantain (*Musa* spp., AAB group) cultivars, 'Bobby Tannap' (BT) and 'Obino l' Ewai' (OL), which are susceptible to black sigatoka, were selected as female parents due to their relatively-high seed fertility (Swennen and Vuylsteke 1993). BT and OL are local plantain cultivars from Cameroon and Nigeria, respectively. The wild non-edible diploid banana *M. acuminata* ssp. *burmannicoides*, clone 'Calcutta 4' (C4), which was originally collected in Burma, was used as

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the male parent due to its high level of resistance to BS. C4 has been considered as a true-breeding line for the few segregating traits studied in *Musa* (Simmonds 1952). Recently, we have been able to extend this conclusion to more than 50 morphological descriptors.

About 600 inflorescences of each plantain cultivar were pollinated with C4, which resulted in the production of 19 500 seeds, of which about 400 were germinated by in-vitro embryo culture. Seedlings obtained from these 3x × 2x crosses were transferred to the nursery and sorted into 2x and 3x or 4x based on their gross morphological characteristics (Simmonds 1948). Chromosome counts confirmed the ploidy level in a sample of the hybrids (Bai K.V., Vandenhout H., Osuji J., personal communications). One hundred-and-one euploid plantain-banana hybrids, referred to as tropical *Musa* plantain hybrids (hereafter TMPx) (Vuylsteke et al. 1993c), were established in selection fields. Aneuploid and hyperploid seedlings, which were easily identified by their abnormal and weak growth, were rogued at the nursery stage.

'C4' and TMPx 1206-2 (a diploid F_1 derived from the cross between the plantain cv 'French Reversion' and 'C4') were self-pollinated. Selfed progenies were evaluated to test the genetic model proposed for BS resistance.

TMPx genotypes established at the Onne station of IITA, which is in the humid forest zone of southeastern Nigeria, were evaluated for BS resistance during the rainy season of 1991 (plant crop) and 1992 (first ratoon). Black sigatoka is ubiquitous at this location, and artificial inoculation was not considered necessary for field screening. The field layout was a completely randomized design with 4–5 replicates (plants) for each TMPx clone. Plants were spaced at 3 m × 2 m, providing a plant density of 1 667 per hectare. Plots were surrounded by the susceptible plantain cultivar 'Agbagba', which served as a reference for host response to BS.

Genotype response to BS was measured by recording the number of the youngest leaf spotted (YLS), counting down from the first (top) open leaf, on medium-sized, non-flowering plants (Vakili, 1968; Stover and Dickson 1970). This was done on an individual plant basis for each TMPx clone. Increasing YLS values indicate the presence of more healthy leaves on the plant and, hence, greater resistance to the fungus. According to Simmonds (1979), selection for lower attack in relation to an appropriate crop maturity, e.g., pre-flowering, works well at an empirical level. YLS was also recorded on randomly-chosen plants of the reference cv 'Agbagba' in four different field sites (two replications of seven plants in two blocks) in order to ascertain the uniformity of disease spread and the minimum number of plants required to evaluate BS resistance. For the latter purpose, the YLS variance index (Y axis) was plotted against the number of plants in an experimental unit (X axis) to determine optimum sample size as indicated by the point of inflection in the graph (Bowman 1989).

Three levels of host response to BS were defined from the mean and standard deviation of the YLS of the susceptible reference cv 'Agbagba'. TMPx clones with a significantly ($P < 0.05$) or highly-significantly ($P < 0.01$) greater number of functional leaves as compared with 'Agbagba' were rated as less susceptible (LS) and partially resistant (PR), respectively, while those which were not different from 'Agbagba' were considered as susceptible (S). The frequency distribution of BS response was tested for homogeneity within and between ploidy levels for OL × C4 and BT × C4 progenies using the log likelihood ratio or G statistics (Sokal and Rohlf 1981). Tests of goodness of fit to expected segregation ratios were performed using χ^2.

Gene-centromere mapping (Mendiburu and Peloquin 1979) was done using half-tetrad analysis for the segregating major BSR locus.

Results

Uniform evaluation trial for black sigatoka

There were no significant differences between blocks and within blocks (reps in blocks) for the BS evaluation parameter YLS in the reference plantain cv 'Agbagba' (Table 1). This suggests a uniform spread of BS in the experimental field. The YLS variance and coefficient of variation decreased, but the mean remained unchanged, as the number of 'Agbagba' plants in each experimental unit increased. The point of inflection in the graph, i.e., the optimum sample size, was observed when the experimental unit was four plants; the coefficient of variation remained unchanged for sample sizes ≥ 4 plants (Table 2).

The mean and standard deviation of YLS in 'Agbagba' was 6.19 and 1.15, respectively, with a range of 4–8 (number of YLS). The scale developed for evaluation of host response of TMPx germplasm to BS disease is shown in Table 3. TMPx clones exceeding the mean YLS of 'Agbagba' by two and three standard deviations were considered as less susceptible and partially resistant, respectively.

Table 1 Analysis of variance for host response to black sigatoka as measured by the number of YLS in the susceptible reference plantain cv 'Agbagba'

Source of Variation	Degrees of freedom[a]	Sum of squares	Mean square	F	*P* level in F table
Blocks (B)	$b-1=1$	1.750	1.750	9.831	> 0.05 ns
Reps/B	$(r-1)b=2$	0.357	0.178	0.126	> 0.05 ns
Residual	$(p-1)br=24$	34.000	1.417		
Total	$pbr-1$	36.107			

Coefficient of variation = 19.2%
[a] b = number of blocks = 2; r = number of reps per block = 2; p = number of plants evaluated per rep = 7

Table 2 Experimental unit size (EUS = number of plants), mean, variance, variance index, and coefficient of variation (CV) for number of YLS evaluated in a uniform evaluation trial of the reference plantain cv 'Agbagba'

EUS	Mean[a]	Variance	Variance index[b]	CV (%)
1	6.2	1.34	1.34	18.7
2	6.2	0.68	1.37	13.2
3	6.3	0.26	0.76	8.0
4	6.2	0.14	0.54	5.9
5	6.3	0.12	0.58	5.5
6	6.3	0.09	0.52	4.7
7	6.2	0.12	0.85	5.7
14	6.1	0.12	1.75	5.8

[a] Mean over EUS
[b] Variance index = variance × EUS

Table 3 Host response to black sigatoka disease according to the evaluation of number of YLS in the susceptible reference plantain cv 'Agbagba'

Host response	Code	'Agbagba' YLS mean[a] + n standard deviation[b]
Susceptible	S	< 8
Less susceptible	LS	8–10
Partially resistant	PR	> 10

[a] Digits were rounded to the nearest integer
[b] Two and three standard deviations added to the 'Agbagba' mean for LS and PR classes, respectively

Host response to black sigatoka in TMPx clones

Most euploid progenies were either 2x or 4x (Vuylsteke et al. 1993b). Only one triploid was recovered in each family. The observed segregation of black sigatoka resistance within the TMPx progenies is presented by family and ploidy level in Table 4. Continuous distributions from susceptible to varying degrees of partial resistance, but not including hypersensitivity or immunity, were observed in both families and ploidies. The response to BS of resistant TMPx genotypes involved slower or delayed disease development (Mobambo et al. 1993). This resulted in less leaf-spot damage at all stage of plant growth when compared with the susceptible TMPx hybrids and reference plantain. The triploid TMPx hybrids showed a response similar to that of 'Agbagba' and were thus considered susceptible. There was no genotype-by-environment interaction for BS response in the TMPx genotypes evaluated over the 2 years at Onne, i.e., non-significant change in BS response was observed in the progenies.

Within each ploidy level, the frequency distributions of YLS in the OL × C4 and BT × C4 families were not different according to the G statistics test (Table 4). This indicated that the two plantain cvs BT and OL were genetically similar for response to black sigatoka.

A significant dosage effect, resulting in an increased level of resistance, was observed in the 4x progenies (Table 4), thus, partially-resistant TMPx clones were more frequent among 4x (52%) than in 2x (21%) types.

Table 4 Segregation of host response to black sigatoka among 2x and 4x plantain-banana progenies obtained from crosses of the susceptible 3x plantain cultivars 'Obino l'Ewai' (OL) and 'Bobby Tannap' (BT) with the wild 2x banana 'Calcutta 4' (C4)

Cross	PR[a]	LS	S	G-stats[b]
4x				
OL × C4	8	3	2	
BT × C4	3	2	3	
Total 4x	11	5	5	1.56 ns
2x				
OL × C4	6	7	7	
BT × C4	10	23	25	
Total 2x	16	30	32	1.41 ns
4x vs 2x				7.81*

[a] PR = partially resistant; LS = less susceptible; S = susceptible
[b] ns, nonsignificant or *, siginificant at $P = 0.05$, respectively (2 *df*)

Genetics of black sigatoka resistance at the 2x level

The variation in BS reaction among the 2x progenies can be explained by the segregation of at least three independent loci having different effects on plantain response to BS. The observed results fitted either a disomic (1PR:3LS:4S) or a trisomic (8PR:10LS:9S) test-cross segregation ratio for simplex plantain parents (Table 5). The model consists of a major locus BS_1 (dominant allele confers BS susceptibility) and two additional indepent loci (bs_2 and bs_3) with additive effects for BS resistance. Diploids with partial resistance are the result of homozygosity for the favourable alleles in the three loci (bs_1/bs_1, bsr_2/bsr_2, bsr_3/bsr_3). The dominant allele BS_1 is always present in the genotype of susceptible TMPx hybrids. Presence of the bs_1 recessive allele in the homozygous state results in a less susceptible response if one or both of the minor additive *bsi* loci are heterozygous, i.e., the favourable effect of the bsr_i (resistance allele) is counterbalanced by the negative effect of the bss_i (susceptibility allele) in each *bsi* additive locus.

Table 5 Genotypes, types of gene action, expected segregation ratios (ESR), and χ^2 tests of goodness of fit in 2x and 4x plantain-banana hybrids[a] (*ns*, nonsignificant)

Genotype (n = 2 for 2x, n = 4 for 4x)	Diploids						Tetraploids				
	ESR[b]		Gene action[c]			Phenotype[d]	ESR[b]	Gene action			Phenotype
	d	t	BS_1	bsr_2	bsr_3			BS_1	bsr_2	bsr_3	
$BS_1\_bsr_2\_bsr_3\_$	1	1	D	0	0	S	1	D	0	0	S
$BS_1\_bsr_2{}^n bsr_3\_$	1	2	D	2	0	S	1	D	4	0	LS
$BS_1\_bsr_2\_bsr_3{}^n$	1	2	D	0	2	S	1	D	0	4	LS
$BS_1\_bsr_2\_bsr_3{}^n$	1	4	D	2	2	S	1	D	4	4	LS
$bs_1{}^n bsr_2\_bsr_3$	1	2	R	0	0	LS	1	R	0	0	PR
$bs_1{}^n bsr_2{}^n bsr_3\_$	1	4	R	2	0	LS	1	R	4	0	PR
$bs_1{}^n bsr_2\_bsr_3{}^n$	1	4	R	0	2	LS	1	R	0	4	PR
$bs_1{}^n bsr_2{}^n bsr_3{}^n$	1	8	R	2	2	PR	1	R	4	4	PR
χ^2 test (2 *df*)	5.2 ns	3.6 ns					3.2 ns				

[a] Derived from crosses between a simplex plantain ($BS_1 bs_1^2 bsr_2^2 bass_2 bsr_3^2 bss_3$) and the homozygous recessive 'Calcutta 4' banana ($bs_1^2 bsr_2^2 bsr_3^2$)
[b] Disomic (d) and trisomic (t) expected segregation ratios were tested at the 2x level. The expected segregation ratio at the 4x level is based upon production of SDR 2n eggs and the assumption that segregating loci are between the centromere and the first cross over
[c] D = dominant susceptible (BS_1), R = recessive resistance (bs_1). Each bsr_2 or bsr_3 has a (+ 1) effect and each bss_2 or bss_3 allele a (− 1) effect
[d] S = susceptible, LS = less susceptible, PR = partially resistant

Genetic model for black sigatoka resistance at the 4x level

The genetic model consists of the same three segregating loci, but with a strong dosage effect due to an increased number of the favourable alleles in the case of a resistant response (Table 5). The observed results fitted the expected segregation ratio (4PR:3LS:1S) of this model. TMPx germplasm with four bs_1 alleles are partially resistant, while TMPx with four bsr_2 or bsr_3 favourable alleles but with the BS_1 susceptibility allele are in the less-susceptible group.

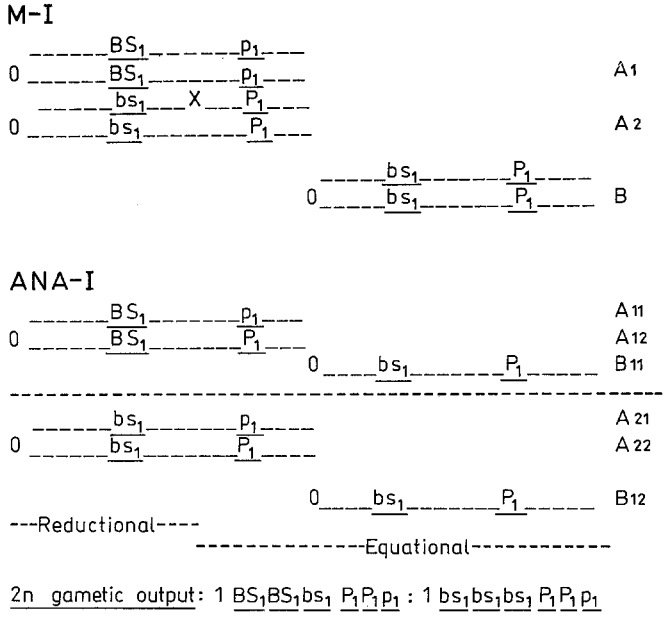
Segregation of BS response within tetraploid families is thought to result from the mode of 2n egg formation (Vuylsteke et al. 1993b), i.e., a second-division restitution (SDR) mechanism allows for the occurrence of first-division segregation during megasporogenesis for the loci involved in the genetic control of BSR. A model of the modified megasporogenesis leading to production of SDR 2n eggs is illustrated in Fig. 1. Results suggest that the BS_1 locus should be very close to the centromere; 52% of 4x progenies were partially resistant (nulliplex for the bs_1 allele), thus the gene-centromere map distance was estimated as '0' (50% – the % of nulliplex) by half-tetrad analysis, which indicates almost complete linkage between the centromere and the BS_1 locus.

Testing the genetic model with S_1 progenies

Selfed progenies of the male parent 'Calcutta 4' (C4) showed phenotypic resemblance to the parental type, which confirms the homozygosity of C4 for BS response and other traits.

TMPx 1206-2 had a YLS of 7.2 and, therefore, was rated as susceptible. Its diploid S_1 progeny segregated for BS response as follows: 1 PR, 4 LS and 29 S. This fits an F_2 segregation ratio for three independent loci ($\chi^2 = 2.98$ ns). The result indicates that the BS susceptibility of TMPx 1206-2 is due to the heterozygosity of this clone for the three BS loci and also confirms that the partially-resistant 2x phenotype is due to the interaction of three independent recessive/additive alleles.

Fig. 1 First-division segregation in second-division restitution (SDR) 2n eggs of plantain (*Musa* spp., AAB group) for a locus close to the centromere (BS_1). No second-division segregation occurs for the P_1 locus, which is located far away from the centromere (at least after the first cross over) due to omission of the second meiotic division. O = centromere; X = cross over



Discussion

The segregation outcome of the OL × C4 and BT × C4 progenies should be similar to that of a test-cross because the male parent (C4) can be considered as a true-breeding line (Simmonds 1952). Phenotypic segregation, with the extremes resembling both parents, occurs in test-crosses due to heterozygosity in one parent. This pattern of segregation confirms the recessive nature of the homozygous parent.

Susceptible phenotypes, resembling the plantain parent, and partially-resistant ones were recovered in the plantain-banana hybrid population. The high level of resistance of C4 was not observed in the segregating progenies, because this type of reaction is masked by the presence of more than one gene (Simmonds 1979) controlling disease resistance.

Shepherd (1990) suggested that resistance to yellow sigatoka (*M. musicola* Leach; YS) in 2x banana hybrids depended, in part, on homozygous recessive genes. He also indicated that it was difficult to discriminate between resistant and susceptible classes without the appearance of plants with intermediate reactions (LS) which were difficult to classify. Shepherd suggested that many genes are involved in the genetic control of YS resistance, with some having a relatively major effect and many more with minor action. This genetic system for YS resistance appears similar to the one reported in this paper for BS resistance.

For many years, *Musa* breeding has been considered to be virtually a matter of breeding superior diploids, because the triploid female genome was believed to be fixed with recombination only possible from the 2x male parent (Rowe 1984; Simmonds 1986; Stover and Buddenhagen 1986; Shepherd 1987). However, the recessive/additive genetic system controlling BS resistance would suggest that utilization of a BSR 2x male parent is not essential to obtain a BSR 4x hybrid. The recessive/additive favourable resistance alleles are present in the 3x plantain genome, but are masked by the susceptibility alleles. The occurrence of first-division segregation during the modified megasporogenesis leading to the formation of SDR 2n eggs in the plantain parent then provides an opportunity to recover BSR 4x TMPx germplasm. However, Utilization of a BSR 2x male parent increases the probability of obtaining a BSR 4x

TMPx and, therefore, BSR 2x male parents should be used as partners in crosses with susceptible seed-fertile plantains.

How do the three loci controlling BS resistance interact?

Potential interactions between the three loci at the 2x and 4x level are illustrated in Fig. 2. In diploids, homozygous bsr_i alleles are not sufficient to produce less-susceptible phenotypes. A homozygous bs_1 genotype, alone or in combination with only one homozygous bsr_i genotype, does not result in a partially-resistant, but rather in a less-susceptible, response. Only diploids with all three favourable alleles in the homozygous state will have a partially-resistant phenotype. Conversely, due to dosage effects (Stern 1929), tetraploids with a homozygous bs_1 genotype will show partial resistance. Similarly, homozygous genotypes for any bsr_i locus have LS phenotypes, even in the presence of the unfavourable BS_1 allele. This means that an increased number of favourable alleles in the 4x TMPx genotypes can overcome the action of the host plant's susceptibility alleles and the virulence genes of the pathogen. Vakili (1968) also explained the enhanced level of yellow sigatoka resistance in 4x banana hybrids as a consequence of the dosage effect of resistance alleles.

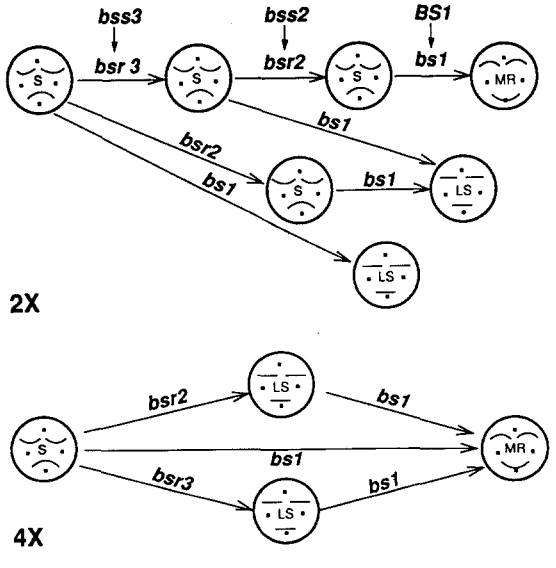
Why are the 3x plantains susceptible?

The simplex genotype proposed for the BS loci in plantains ($BS_1 bs_1^2 bsr_2^2 bss_2 bsr_3^2 bss_3$; see Table 5) agrees with its AAB genomic composition. BS-susceptible and resistant accessions have been reported in *M. acuminata* (A genome), whereas all known *M. balbisiana* accessions (B genome) show high levels of resistance (Tezenas du Montcel 1990). This suggests that the original interspecific cross, which resulted in the production of plantain, was between a heterozygous (at least for the BS loci) AA accession producing 2n eggs and a BB accession with the BSR recessive/additive alleles producing normal n pollen.

The similarity between the BS-susceptible genotypes OL (Nigerian cultivar) and BT (a cultivar from Cameroon) also indicates that somatic mutations, which generated morphological variability in the African centre of plantain diversification (De Langhe 1964), did not occur in the BS loci due to lack of coevolution with the pathogen in Africa.

Plantains are susceptible because they are simplex for the BS_1 locus and have only two copies of the bsr_i alleles. The net favourable effect in the bs_i loci of plantain (= 2) is similar to the diploids which are homozygous for one of the two bs_i loci but heterozygous for the BS_1 locus. The two 3x TMPx hybrids were also susceptible, which suggests that their genotype for the BS_1 locus could be similar to the plantain parent and that the net effect in the bs_i loci should be less than four. No hypothesis can be statistically tested in the group of 3x hybrids due to the small sample size.

Fig. 2 Genetics of host response to black sigatoka disease in plantain/banana (*Musa* spp.). Resistance is under the control of one recessive allele (bs_1) and the modifier gene action of another two independent additive resistance alleles (bsr_2 and bsr_3). S = susceptible reaction, LS = less susceptible, MR = moderately resistant



Field evaluation of BS resistance in the TMPx germplasm

Simmonds (1979, 1991) pointed out that experimental assessment of resistance should be done in the field using a proper experimental design with replication and that resistance should always be recorded on a scale relative to something that has been arbitrarily labelled susceptible, e.g., a standard susceptible cultivar. Simmonds and Wastie (1987) found that simple means of visual scores on arbitrary scales were as good as more elaborate measures for assessing resistance. In our study, visual BS scoring could be done on unreplicated plantings due to the high heritability of resistance and the ubiquity and high inoculum pressure of *M. fijiensis* in the experimental field. Also, appropriate reference cultivars were included in the selection fields as susceptible checks.

Likelihood of durability of horizontal BS resistance in TMPx germplasm

Simmonds (1979, 1991) suggested that the salient features of horizontal resistance are: (1) polygenic inheritance (but need not be), (2) continuous distributions from susceptibility to varying degrees of resistance in segregating progenies, (3) less-susceptible or partially-resistant response in the 'resistant' host plant rather than immunity, i.e., the typically slows the progress of a

disease without inhibiting its initiation, (4) pathotype non-specificity, (5) durability over time due to a low genotype-by-environment interaction effect, (6) diverse components of 'less disease' such as infection resistance, slow lesion development and reduced sporulation, and (7) critical demonstration by genetic analysis. Features (2), (3), (5), and (6) have been observed for the response of selected TMPx germplasm to black sigatoka disease.

Horizontal resistance is not necessarily under polygenic control and, as Simmonds (1991) pointed out, it is likely that few genes confer such resistance. Simmonds (1979) also suggested that complex genetic systems can be broken down into specific components given good environmental control of infection and sufficient study. When considering the gene-for-gene hypothesis (Flor 1971), a host plant resistance system based on recessive alleles may prove difficult to break down because it would require a dominant mutation in the major virulence locus of the pathogen. Dominant mutations are rare (Simmonds 1979), which will enable durability of the recessive BS resistant system. For example, the monogenic recessive resistance to Victoria blight (*Helminthosporium victoriae*) in oats (Day 1974) has been described as a very stable and durable type of horizontal resistance (Simmonds 1979) because the resistance genes apparently cannot be matched by the virulence genes of the pathogen.

Genetic control of BS resistance by at least three loci presents a problem to *M. fijiensis*, because the chance of obtaining the right combination of virulence alleles in a single pathotype is much smaller than the chance of securing this if only a single virulence locus were involved. Therefore, BS resistance in IITA's TMPx germplasm, which is based on a recessive/additive oligogenic model, can be considered as an example of durable horizontal resitance.

Mechanisms of BS resistance in relation to the proposed genetic model

Different mechanisms of BS resistance have been suggested by biochemical and morphological comparison of cultivars with a range of responses to the disease. Lepoivre and Acuna (1990) indicated that host resistance to pathogens could be due to (1) the synthesis of antimicrobial compounds, e.g., phytoalexins, (2) the production of lignin or suberin which arrests penetration of the pathogen, and (3) resistance to phytotoxins produced by the pathogen. Japayal and Mahadevan (1968) reported that there was a correlation between the polyphenolic content of healthy banana tissues and resistance against leaf-spot pathogens. Sallé et al. (1990) also observed that a BS-resistant *Musa* cultivar had higher polyphenolic content in healthy leaf tissue than a BS-susceptible cultivar. Mourichon et al. (1990) found that leaf tissue extracts of resistant cultivars were fungitoxic and that the active substances therein could be a component of BS resistance. Production of a phytoalexin may result from the action of a major gene, such as bs_1, in partially-resistant TMPx hybrids.

Polyphenols produced in response to infection by BS may have a fungicidal or fungistatic effect and could be a component of some hypersensitive inhibition of infection (Simmonds 1979). This type of vertical resistance, which delays the start of the disease, disappears when a virulent pathotype becomes prevalent in the pathogen population. The outcome of the host-pathogen interaction is then determined by the genes involved in horizontal resistance (Simmonds 1979).

Low stomata density and increased epicuticular wax in leaves of resistant cultivars could provide additional mechanisms of BS resistance. Vazquez et al. (1990) found variation in stomata density between the 3x cultivars '*Pelipita*' (resistant ABB cooking banana), 'False Horn' (susceptible AAB plantain), and 'Grande Naine' (highly-susceptible AAA banana). This suggests that this trait may be under additive genetic control. Also, increasing ploidy levels generally show decreasing stomata density (Simmonds 1948; Borges 1971), which could explain the dosage effect for BS resistance observed in the 4x TMPx germplasm.

Epicuticular wax could also be involved in host response to BS (Vazquez et al. 1990) as it may reduce the accumulation of moisture on emerging leaves and thereby retard the establishment and germination of fungal spores. Waxiness of the *Musa* pseudostem, which consists of overlapping leaf sheaths, has been found to be due to a recessive *wx* gene (Ortiz and Vuylsteke, in preparation). The degree of expressivity for waxiness seems to be under polygenic control and could be increased by additional copies of the *wx* allele in the 3x plantain or its derived 4x TMPx hybrids. The two morphological BS resistance/evasion mechanisms, stomatal density and waxiness, could be either under the independent genetic control of the proposed bsr_i alleles or controlled by genes tightly linked to the bs_i loci.

M. fijiensis, the causal agent of black sigatoka, produces phytotoxins that have recently been isolated and identified (Lepoivre and Acuna 1990; Upadhyay et al. 1990; Stierle et al. 1991). The most abundant and phytotoxic of these compounds is 2, 4, 8-trihydroxytetralone, a melanin-shunt-pathway metabolite, which induces necrotic lesions in susceptible banana cultivars. The production of melanin has been suggested as an important determinant of pathogenicity (Stierle et al. 1991). The chemical structure of 2, 4, 8-trihydroxytetralone should be compared with that of the polyphenols produced by BS-resistant cultivars to determine if complementarity exists between the products coded by the BSR allele in the host plant and the BS virulence allele of the pathogen. In this regard, we have initiated the molecular characterization of BS resistance, evidently a complex trait, by using restriction fragment length polymorphisms (RFLPs) and random amplified polymorphic DNA (RAPDs) for marker analysis in the segregating 4x and 2x populations of OL × C4 and BT × C4 test-crosses.

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