Technical paper

Breeding and evaluation of *Musa* hybrids resistant to *Fusarium oxysporum* f. sp. *cubense* race 1

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Breeding and evaluation of *Musa* hybrids resistant to *Fusarium oxysporum* f. sp. *cubense* race 1.

Abstract — **Introduction**. Wilt caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*) is the most important disease of banana worldwide, causing severe yield losses in commercial and local consumption cultivars. Chemical control is currently the most used method to manage the Fusarium wilt of banana, although it is toxic, expensive and dangerous. Therefore, control through genetic improvement is widely encouraged. Hence, breeding was carried out to develop banana hybrids with putative resistance. **Materials and methods**. Hybridization of identified resistant diploids with commercial triploids and tetraploids was carried out; it resulted in development of 22 hybrids with improved agronomic characters. In a second step, screening of these hybrids for resistance to *Foc* race 1 was attempted using the double cup method of challenge inoculation with the pathogen in the roots of the hybrids at optimum level. Host responses of the susceptible and resistant hybrids were examined under greenhouse conditions through biochemical and isozyme analysis. **Results**. Six hybrids among the 22 hybrids with improved agronomic characters were selected based on their yield and they were evaluated for resistance. Three of them were resistant to *Foc* and the others were susceptible. The resistance mechanism involving the relation of enzymes such as peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine lyase (PAL), and biochemicals such as phenols, proline and lignin showed relatively higher activity in resistant hybrids and parents than susceptible ones. Isozyme analysis of PO and PPO carried out using selected resistant and susceptible hybrids revealed the induction of specific isoforms in the resistant hybrids upon challenge inoculation. **Conclusion**. Three hybrids were identified as potential hybrids with good yield and resistance to *Foc* race 1.

India / *Musa* / *Fusarium oxysporum* / disease control / disease resistance / plant breeding / defence mechanisms / enzymatic analysis / polyphenol oxidase / peroxidases / phenylalanine ammonia lyase

Multiplication et évaluation d'hybrides de bananiers résistants à la race 1 de *Fusarium oxysporum* f. sp. *cubense*.

Résumé — **Introduction**. Le wilt causé par *Fusarium oxysporum* f. sp. *cubense* (*Foc*) est la maladie du bananier la plus importante au niveau mondial ; il provoque de graves baisses de rendement dans les cultivars comestibles commerciaux et locaux. Le contrôle chimique est actuellement la méthode la plus utilisée pour lutter contre ce wilt, bien qu'il soit toxique, cher et dangereux. Par conséquent, la voie de l'amélioration génétique est vivement encouragée. Un programme de sélection a donc été entrepris pour développer des hybrides de banane potentiellement résistants. **Matériel et méthodes**. Une hybridation de diploïdes identifiés comme résistants avec des cultivars triploïdes et tétraploïdes a été effectuée ; elle a permis de développer 22 hybrides présentant des caractères agronomiques améliorés. Dans une deuxième étape, un tri de ces hybrides vis-à-vis de leur résistance à la race 1 du *Foc* a été entrepris en utilisant une méthode spécifique. Les réponses au parasite des hybrides sensibles et résistants ont été étudiées sous serre par des analyses biochimiques et d'isozymes. **Résultats**. Six hybrides parmi les 22 sélectionnés pour leurs caractères agronomiques améliorés ont été retenus pour leur rendement ; ils ont été évalués pour leur résistance au *Foc*. Trois hybrides se sont révélés résistants et les autres ont été sensibles. Le mécanisme de résistance impliquant la relation d'enzymes comme la peroxydase (PO), l'oxydase de polyphénol (PPO) et la lyase phénylique d'alanine (PAL), ainsi que des composés biochimiques comme les phénols, la proline et la lignine ont montré une activité relativement plus élevée dans les hybrides et les parents résistants que dans les plants sensibles. L'analyse des isozymes du PO et du PPO effectuée en utilisant des hybrides sensibles et résistants a révélé une induction d'isoformes spécifiques parmi les hybrides trouvés résistants à partir de tests d'inoculation. **Conclusion**. Trois hybrides ont été identifiés qui présentent potentiellement de bons rendements et une résistance à la race 1 du *Foc*.

Inde / *Musa* / *Fusarium oxysporum* / contrôle de maladies / résistance aux maladies / amélioration des plantes / mécanisme de défense / analyse enzymatique / polyphénol oxydase / péroxydase / phénylalanine ammonia lyase

1. Introduction

Banana and plantains (*Musa* spp. L.) are the second largest fruit crops in the world; they are important staple foods in tropical America, Asia and the Pacific. Cultivated bananas are sterile triploid varieties evolved from intra- and inter-specific crosses of two diploid species: *M. acuminata* Colla., donor for the A genome, and *M. balbisiana* Colla., donor for the B genome. Banana breeding started in Trinidad in 1922 [1] and in India in 1949 [2]. The driving force of this breeding program was the development of improved *Fusarium oxysporum* f. sp. *cubense* (*Foc*)-resistant germplasm for export trade [3]. Yield loss in South India alone due to *Foc* was estimated to range from 2–90% [4]. Prominent symptoms of infection with *Foc* are yellowing of the foliage, splitting of the pseudostem base and vascular discoloration. The most popular cultivars grown in India, which include Rasthali (Silk), Pisang Awak, Poovan (Mysore), Nendran (French Plantains) and Virupakshi (Pome), are susceptible to *Foc* race 1.

Traditional genetic improvements must come from potential and improved diploids, which can be further crossed with commercial varieties/hybrids to develop new hybrids [5].

In India, diploid breeding was initiated by hybridizing Matti (AA) as the female parent with diploid male parents like Anaikomban, Pisang Lilin, Tongat and Namarai, as they possessed resistance to *Foc* race 1. In continuation of this breeding program, the current study was framed to develop resistant hybrids by hybridization of identified potential diploids like Pisang Lilin and Anaikomban (resistant to *Foc*) with commercial triploids (Pisang Awak), to develop primary tetraploids. The above-mentioned diploids were also hybridized with a synthesized tetraploid (H-02-32) to develop secondary triploids. The resistance of the newly developed hybrids to Fusarium wilt (race 1) was tested along with a study of their biochemical constituents, in response to inoculation with *Foc*.

2. Materials and methods

2.1. Synthesis of hybrids

Unopened anthers, just prior to dehiscence, were collected from the inflorescence and the pollen smeared over the surface of receptive stigma of female flowers for production of new hybrids [6]. Hybridization was attempted using H201, H-02-031, H-02-32, Peykunnan and Baro Cemsa as female parent and the resistant diploids like Pisang Lilin and Erachi Vazhai as male parent. The new *Musa* hybrids obtained were assessed for ploidy level using stomatal density and flow cytometry analysis [7].

2.2. Preparation of Foc cultures

Race 1 of *Foc* was isolated from the Fusarium wilt (race 1; VCGs 0124/5)-affected banana cv. Rasthali (Silk, AAB) and, to avoid bacterial contamination, it was cultured in petri dishes on potato dextrose agar medium (PDA) amended with 100 $\mu g \cdot g^{-1}$ of streptomycin sulfate. The dishes were incubated at 28 °C for 5 d, then they were observed for the presence of *Foc* based on the description given by Snyder and Hansen [8]. The pathogen was multiplied in PDA broth, which was incubated at room temperature and shaken twice a day for 7 d before being filtered through two-layered cheesecloth.

2.3. Double cup method

Resistance of potential hybrids was confirmed by screening them with susceptible hybrids (resulting from the pot culture experiment) and parents, following the double cup method. Shoot tips of the hybrids were tissue-cultured on Murashige and Skoog medium amended with 1–5 $mg \cdot L^{-1}$ benzylaminopurine (BAP) and 1–3 $mg \cdot L^{-1}$ of indole-3-acetic acid (IAA). The plantlets were transferred to sterilized soil medium in trays for initial hardening under greenhouse conditions. The roots of the hardened plants

Table I.
Scoring scale used for evaluation of the resistance of parthenocarpic *Musa* hybrids to *Fusarium oxysporum* f. sp. *cubense* (*Foc*) [9].

Scale	Leaf symptom index	Rhizome discoloration index
1	No streaking or yellowing of leaves	No discoloration of the stellar region
2	Slight streaking / light yellowing of lower leaves	Discoloration at juncture of root and rhizome
3	Streaking / yellowing of most of lower leaves	5% stellar region discolored
4	Extensive streaking / yellowing of leaves	6–20% stellar region discolored
5	Dead plant	21–50% stellar region discolored
6	–	More than 50% stellar region discolored
7	–	Discoloration of entire rhizome
8	–	Dead plant

Translation of disease severity index scales regarding *Musa* hybrid resistance to *Foc*. Disease severity index was measured with the ratio: [∑(number of scale × number of seedlings in that scale) / ∑ (number of treated seedlings)].

Disease severity index scale for leaf symptom index	Disease severity index scale for rhizome discoloration index	Translation
1.0	1.0	Resistant
Between 1.1 and 2.0	Between 1.1 and 3	Tolerant
Between 2.1 and 3.0	Between 3.1 and 5	Susceptible
Between 3.1 and 4.0	Between 5.1 and 8.0	Highly susceptible

were inoculated with *Foc* by immersing in the appropriate conidial suspension (10^4 conidia·mL^{-1}) of *Foc* race 1 for 2 h before being tagged and replanted into a small disposable cup with four drainage holes at the base. Through the four drainage holes, selected healthy white roots were allowed to pass into a suitably sized plastic tumbler partially filled with Hoagland's solution to partially cover the root zone. The evaluation for resistance was performed 3 months after inoculation. A non-inoculated control was maintained under each hybrid and parent used in the study for simultaneous evaluation.

Final evaluation was made based on the leaf symptom index (LSI) and rhizome discoloration index (RDI) used by Brake *et al.* [9] (*table I*). Disease severity index (DSI) was measured with the ratio: [∑(number of scale × number of seedlings in that scale) / ∑ (number of treated seedlings)].

2.4. Biochemical assay

The activity of defense-related enzymes, *viz.*, peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL), and the content of phenolics and lignin in the roots were determined for each replicate at the end of the third month prior to uprooting for scoring wilt resistance. The total phenolic content in the roots was estimated using Folin Ciocalteau reagent and spectrophotometrically measured at 660 nm wavelength; it was expressed as µg·g^{-1} root [10]. The lignin content in roots, expressed as %, was gravimetrically estimated following the method of Chesson [11], using a mixture of 5 mL of concentrated H_2SO_4 and 50 mL of HCl for 16 h at 25 °C in a shaker.

2.5. Enzyme extraction

One gram of root sample per replicate was homogenized with 2 mL of 0.1 M sodium

phosphate buffer (pH 7.0) at 4 °C. The homogenate was centrifuged for 20 min at 8000 *g*. The supernatant was used as crude enzyme extract for assaying PO and PPO. PO activity was assessed according to Hammerschmidt *et al.* [12]. The reaction mixture (3 mL) consisted of 0.25% (vol. / vol.) guaiacol in 10 mM potassium phosphate buffer (pH 6.9) containing 10 mM hydrogen peroxide. PPO was assessed using the modified method of Mayer *et al.* [13], using 0.1 M phosphate buffer (pH 6.5) and 0.01 N catechol along with enzyme extract. The PAL assay was conducted according to the method described by Ross and Sederoff [14] using 500 µL of borate buffer and 600 µL of 12 $mM \cdot L^{-1}$ phenylalanine along with 400 µL enzyme extract as reaction mixture. The enzyme assays of PO, PPO and PAL were measured spectrophotometrically at (470, 495 and 290) nm, respectively.

2.6. Isozyme analysis of the enzyme PO and PPO

For native anionic polyacrylamide gel electrophoresis, resolving gels of 8% acrylamide concentration and stacking gels of 4% acrylamide concentration were prepared. The electrophoresis conditions were maintained at 4 °C with 80 V for 3 h. For estimation of PO, the gels were incubated in darkness in a solution containing 100 mg benzidine dissolved in 0.5 mL of acetone, in 50 mL of acetate buffer for a period of 30 min after electrophoresis. Drops of 30% H_2O_2 were added with constant shaking until the bands appeared [15]. For PPO, after electrophoresis for 3 h with 80 V at 4 °C, the gel was equilibrated for 30 min in 0.1 M phosphate buffer (pH 7.0) containing 0.1% -phenylene diamine, followed by 10 mM catechol in 0.1 M phosphate buffer (pH 7.0). Gentle agitation followed by addition of catechol resulted in the appearance of dark brown discrete protein bands [16].

2.7. Statistical analysis

The experiment was carried out in a completely randomized design. Each hybrid was replicated four times with 10 plantlets under each replication. All data were analyzed using SPSS 11.5 statistical software. The variability between the hybrids for the different biochemical and yield characters was analyzed using ANOVA by the LSD method, while the relationship between the biochemical parameters and the resistance was measured using multiple stepwise regression models.

3. Results

3.1. Development of resistant hybrids

A total of 4839 crosses were performed which resulted in the production of 893 seeds, of which 101 germinated. Twenty-two hybrids were selected for further evaluation because of their parthenocarpic nature (*table II*), whilst the non-parthenocarpic hybrids were discarded. Six of these hybrids, along with two susceptible parents, were selected based on improved yield for screening against *Foc*.

Screening of six hybrids and two parents using the double cup method showed that three hybrids (NPH-02-01, H-03-13 and H-03-19) displayed resistance, while two hybrids were highly susceptible and one hybrid was classified as being susceptible (*table III*).

3.2. PO, PPO and PAL activities

Spectrophotometric analysis showed significantly higher PO, PPO and PAL activities in resistant genotypes than in the susceptible genotypes in both the inoculated and non-inoculated control (*table III*). However, the control (non-inoculated) hybrids invariably showed lower enzyme activity when compared with the *Foc*-inoculated hybrids. The PO activity in the resistant hybrids inoculated with *Foc* showed significant variation, ranging from 8.34 $\Delta A_{470} \cdot min^{-1} \cdot g^{-1}$ fresh weight for NPH-02-01 to 12.67 $\Delta A_{470} \cdot min^{-1} \cdot g^{-1}$ fresh weight for H-03-19. Conversely, in susceptible hybrids inoculated with *Foc*, there was no conspicuous difference in PO activity. The activity of PPO varied significantly from 0.92 $\Delta A_{470} \cdot min^{-1} \cdot g^{-1}$ fresh weight to

Table II.
List, ploidy and yield of twenty-two hybrids selected, because of their parthenocarpic nature, for further evaluation regarding their resistance to *Fusarium oxysporum* f. sp. *cubense* (*Foc*).

No.	Hybrid	Ploidy level	Parentage	Yield (kg per plant)
1	H-03-01	Triploid	Burro Cemsa × Pisang Lilin	17.00
2	H-03-02	Diploid	H-02-32 × Pisang Lilin	4.00
3	H-03-03	Diploid	(Nivedhyakadali × Pisang Lilin) × Pisang Lilin	9.00
4	H-03-04	Tetraploid	NPH-02-03 × Pisang Lilin	6.00
5	H-03-05	Tetraploid	Peykunnan OP (open pollinated hybrid)	11.00
6	H-03-06	Diploid	H-02-32 × Pisang Lilin	4.00
7	H-03-07	Tetraploid	Peykunnan OP (open pollinated hybrid)	3.50
8	H-03-08	Diploid	H-02-32 × Pisang Lilin	4.50
9	H-03-09	Tetraploid	Peykunnan × Erachi Vazhai	9.00
10	H-03-10	Tetraploid	Peykunnan × Pisang Lilin	6.00
11	H-03-11	Tetraploid	H-02-32 × Pisang Lilin	13.00
12	H-03-12	Tetraploid	Peykunnan × Pisang Lilin	8.00
13	H-03-13	Tetraploid	Peykunnan × Erachi Vazhai	21.00
14	H-03-14	Tetraploid	H-02-31 × Pisang Lilin	5.50
15	H-03-15	Triploid	H-02-32 ×Pisang Lilin	14.50
16	H-03-16	Tetraploid	Peykunnan × Pisang Lilin	10.00
17	H-03-17	Tetraploid	Peykunnan × Pisang Lilin	13.00
18	H-03-18	Tetraploid	H-02-31 × Pisang Lilin	6.00
19	H-03-19	Tetraploid	Peykunnan × Erachi Vazhai	24.50
20	H-03-20	Tetraploid	H-02-31 × Pisang Lilin	5.50
21	H-03-21	Tetraploid	Peykunnan OP (open pollinated hybrid)	6.00
22	NPH-02-01	Diploid	H201 × Anai Komban	15.00
Critical difference (5%)				2.14
Standard error of deviation				1.05

1.10 $\Delta A_{470} \cdot min^{-1} \cdot g^{-1}$ fresh weight among the resistant hybrids, and from 0.23 $\Delta A_{470} \cdot min^{-1} \cdot g^{-1}$ fresh weight to 0.40 $\Delta A_{470} \cdot min^{-1} \cdot g^{-1}$ fresh weight in susceptible hybrids. The PAL activity ranged from (17.89 to 22.32) $nmol \cdot min^{-1} \cdot mL^{-1}$ in resistant hybrids, and from (3.35 to 4.08) $nmol \cdot min^{-1} \cdot mL^{-1}$ among susceptible hybrids (*table III*).

3.3. Phenols and lignin

Biochemical activities in the roots of hybrids and parents inoculated with *Foc* (*table IV*) showed significant variations among the inoculated, non-inoculated, control and parents for total phenols and lignin content between resistant and susceptible hybrids. Additionally, the resistant genotypes had relatively higher total phenolic content [(556.50 to 616.50) $\mu g^{-1} \cdot g^{-1}$] and lignin [(0.95 to 1.28)%] when compared with the susceptible hybrids. The hybrids NPH-02-01, H-02-08, H-03-19, and H-03-13 had higher activity of phenols and lignin with higher yield and quality parameters.

3.4. Regression analysis of the biochemical markers associated with resistance

Analysis of resistance using stepwise regression resulted in the identification of two qualitative parameters, lignin content and PPO activity (*table V*), showing negatively

Table III.
Enzyme activities in roots and resistance status of *Musa* hybrids both inoculated with *Fusarium oxysporum* f. sp. *cubense* (*Foc*) and non-inoculated.

Hybrid	Peroxidase (PO) (Δ A_{470}·min^{-1}·g^{-1} fresh weight)		Polyphenol oxidase (PPO) (Δ A_{470}·min^{-1}·g^{-1} fresh weight)		Phenylalanine ammonia-lyase (PAL) (nmol·min^{-1}·mL^{-1})		Score		Resistance status regarding *Foc*
	Inoculated	Control	Inoculated	Control	Inoculated	Control	Leaf symptom index	Rhizome discolouration index	
H-03-01	3.45	2.88	0.40	0.32	3.35	2.05	4.5	6.5	Highly susceptible
H-03-02	2.35	2.21	0.35	0.26	3.45	3.17	4.0	5.0	Susceptible
H-03-03	2.80	1.93	0.23	0.20	5.22	4.08	5.0	7.0	Highly susceptible
H-03-13	9.65	9.00	0.99	0.90	21.66	16.59	1.0	1.0	Resistant
H-03-19	12.67	11.25	1.10	0.93	22.32	15.80	1.0	1.0	Resistant
NPH-02-01	8.34	7.37	0.92	0.90	17.89	13.39	1.0	1.0	Resistant
Red Banana	2.89	2.35	0.38	0.26	4.76	3.05	5.0	7.0	Highly susceptible
Robusta	2.21	1.80	0.36	0.22	5.03	3.17	4.5	6.0	Highly susceptible
Critical difference (5%)	1.38	1.07	0.31	0.28	1.97	2.14	–	–	–

Table IV.
Biochemical activities in roots of both inoculated and non-inoculated *Musa* hybrids with their status of resistance to *Fusarium oxysporum* f. sp. *cubense* (*Foc*).

Hybrid	Total phenols (µg·g^{-1} fresh weight)		Lignin (%)		Resistance status regarding *Foc*
	Inoculated	Control	Inoculated	Control	
H-03-01	259.00	233.00	0.51	0.36	Highly susceptible
H-03-02	355.00	249.50	0.46	0.30	Susceptible
H-03-03	308.00	239.00	0.32	0.26	Highly susceptible
H-03-13	589.00	556.50	1.09	0.95	Resistant
H-03-19	616.50	581.00	1.28	1.15	Resistant
NPH-02-01	620.00	578.50	1.39	1.04	Resistant
Red Banana	256.00	248.00	0.24	0.20	Highly susceptible
Robusta	241.50	228.50	0.30	0.25	Highly susceptible
Critical difference (5%)	51.89	41.13	0.23	0.24	–

highly significant regression with the resistance. This confirms the existence of correlation between the two qualitative parameters (lignin content and PPO activity) and *Foc* resistance. The coefficient of determination, *i.e.*, the adjusted R^2 value of lignin content alone was 0.715 (71%) and that of the combination of lignin content and PPO activity was 0.768 (76%).

3.5. Isozyme analysis

Isozyme analysis showed induction of PO and PPO isoforms in highly resistant seedlings after inoculation. The induction pattern of PO in banana hybrids inoculated with *Foc* and the non-inoculated control indicated the expression of three isoforms, *viz.*, PO1, PO2 and PO3 (*figure 1*). The

Table V.
Regression analyses of the two biochemical parameters, lignin content and PPO activity in *Musa* hubrids, with resistance to *Fusarium oxysporum* f. sp. *cubense* (*Foc*) coefficients (dependent variable: score).

Model	Biochemical parameter	Unstandardized coefficients		Standardized coefficients	t
		B	Standard error	Beta	
1	Constant	6.086	0.258	–	23.571
	Lignin content	– 3.977	0.331	– 0.848	– 11.997
2	Constant	6.256	0.237	–	26.346
	Lignin content	– 2.790	0.438	– 0.595	– 6.371
	PPO activity	– 1.961	0.529	– 0.347	– 3.711

Model summary

Model	*R*	R square	Adjusted R square	Durbin Watson residual
1	0.848[a]	0.720	0.715	0.336
2	0.881[b]	0.776	0.768	–

[a] Predictors: (constant), Lignin content.

[b] Predictors: (constant), PPO activity.

unique expression of the PO2 isoform was expressed only in the hybrids inoculated with *Fusarium*. However, there was no difference in the banding pattern of resistant and susceptible hybrids. Studies on the expression pattern of PPO (*figure 1*) revealed the expression of five isoforms, *viz.*, PPO1, PPO2, PPO3, PPO4 and PPO5. In the non-inoculated control (lanes 7 to 12) only two isoforms were exhibited (PPO 3 and PPO 5). In the *Foc*-inoculated hybrids, expression of three isoforms (PPO1, PPO2 and PPO4) was found typically in only resistant hybrids (lanes 1, 4 and 5), while it was not expressed in susceptible hybrids (lanes 2, 3 and 6).

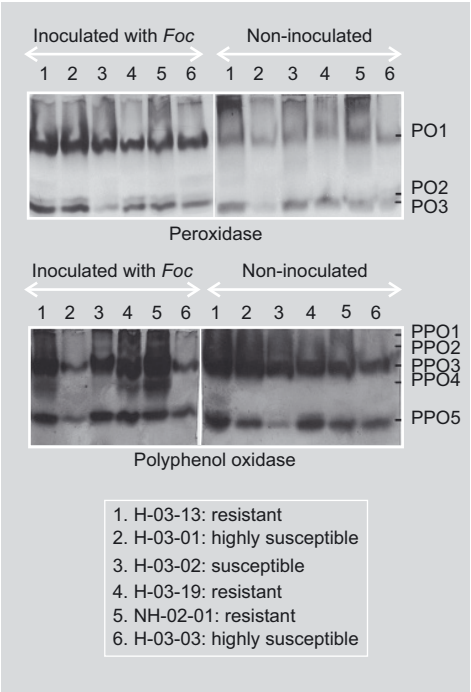

Figure 1.
Induction of isoforms of peroxidase (PO) and polyphenol oxidase (PPO) in six *Musa* hybrids inoculated with *Fusarium oxysporum* f. sp. *cubense* (*Foc*) compared with non-inoculated banana hybrids.

4. Discussion and conclusion

Poor fertility and poor viability of seeds is common in hybridization. This may be due to the presence of structural hybridity and chromosomal aberrations [17]. Analysis of hybrids for inheritance revealed that resistant diploid, triploid and tetraploid hybrids had either Pisang Lilin or Anaikomban as one of the parents. However, certain hybrids, *viz.*, H-03-13 and H-03-19, exhibited resistance despite the fact that both the parents used were susceptible. Recovery of resistant genes from a [susceptible × susceptible] combination may be due to the transgressive segregation of the hybrids because of the heterozygous nature of the parents used in crossing [18].

Resistant hybrids to *Foc* usually exhibit higher PO and PPO activities [19]. The role of peroxidases has been cited in a number of defense mechanisms against the invading pathogens such as hypersensitive response [20], the formation of papilla [21] and lignin [22] in host plant cells. The PPO oxidizes phenols into highly toxic quinones and is hence considered to play an important role in disease resistance, particularly those affecting the tissues [23]. About three to ten times higher PPO activity was noticed in resistant hybrids when compared with susceptible hybrids [24]. These results suggest the possible involvement of acidic isoforms of PO in the host response, as reported by Miyazawa *et al*. [25]. Fungal infection results in *de novo* synthesis of PAL [26]. PAL is the first enzyme to signal the onset of fungal infection, even before the formation of the fungal hyphae [27]. This suggested the strong relation between the enzymes and resistance to *Foc*. Enzyme assay studies showed that the time course pattern of peroxidase accumulation was different in resistant and susceptible pearl millet seedlings [28]. The resistance mechanism involving the role of phenols, PAL, oxidative enzymes such as PO, PPO, superoxidase dismutase, catalase and pathogenesis-related proteins (PR proteins) such as chitinase was reported to show relatively higher activity in resistant plants than in susceptible ones [29].

Most plants synthesize toxic compounds such as phenols, proline and lignin during normal development, and their role in the resistance mechanism has been reported earlier by many authors [30, 31]. These can be used as markers for selection of resistant genotypes. Tolerant plants, when subjected to biotic stress, showed elevated levels of free phenolics and contained more lignin [32].

In regression analysis, the lower percent of the Durbin Watson residual of 0.336 (30%) indicated the fitness of the model. A similar correlation has been established using a lytic assay as a biochemical tool to differentiate pearl millet cultivars for downy mildew disease resistance [33]. The results of the isozyme analysis for PO and PPO showed the possible involvement of acidic isoforms of PPO in host resistance, which was also reported by Miyazawa *et al*. [25]. This also signifies that these hybrids may express their resistance mechanism through this enzyme.

In conclusion, the overall evaluation of the 22 parthenocarpic *Musa* hybrids led to the identification of the hybrids NPH-02-01, H-03-05, H-03-13, H-03-15, H-03-17 and H-03-19 with high yield potential as well as increased resistance to wilt, when compared with conventional cultivars.

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Multiplicación y evaluación de híbridos de bananos resistentes a la raza 1 de *Fusarium oxysporum* f. sp. *cubense*.

Resumen — Introducción. El wilt causado por *Fusarium oxysporum* f. sp. *cubense* (*Foc*) es la enfermedad del banano más importante a nivel mundial; provoca importantes descensos de rendimiento en los cultivares comestibles comerciales y locales. Actualmente, el método más empleado para luchar contre ese wilt es el control químico, a pesar de que sea tóxico, caro y peligroso. Consecuentemente, se impulsa fuertemente la vía de mejora genética. Por ello, se ha iniciado un programa de selección para desarrollar híbridos de banano potencialmente resistentes. **Material y métodos**. Se efectuó una hibridación de diploides identificados como resistentes con cultivares triploides y tetraploides; ésta permitió desarrollar 22 híbridos que presentaban caracteres agronómicos mejorados. En una segunda etapa, se llevó a cabo una selección de estos híbridos con respecto a su resistencia a la raza del *Foc* empleando para ello un método específico. Se estudiaron las respuestas al parásito de híbridos sensibles y resistentes en invernadero mediante análisis bioquímicos y de isozimas. **Resultados**. Se retuvieron para su rendimiento seis híbridos entre los 22 seleccionados por sus respectivos caracteres agronómicos mejorados. Se evaluaron en cuanto a su resistencia al *Foc*. Tres híbridos resultaron ser resistentes y los otros fueron sensibles. El mecanismo de resistencia que implica la relación de encimas como la peroxidasis (PO), la oxidasis de polifenol (PPO) y la liasa fenólica de alanina (PAL), así como otros compuestos bioquímicos como los fenoles, la prolina y la lignina, mostraron una actividad relativamente más elevada en los híbridos y en los padres resistentes que en otras plantas sensibles. El análisis de los isozimas del PO y del PPO efectuados mediante el empleo de híbridos sensibles y resistentes mostró una inducción de isoformas específicas entre los híbridos calificados como resistentes a partir de tests de inoculación. **Conclusión**. Se identificaron tres híbridos que presentan potencialmente buenos rendimientos así como una resistencia a la raza 1 del *Foc*.

India / *Musa* / *Fusarium oxysporum* / control de enfermedades / resistencia a la enfermedad / peroxidases / fitomejoramiento / mecanismos de defensa / análisis enzimático / polifenol oxidasa / peroxídasas / amonio fenilalanina liasa