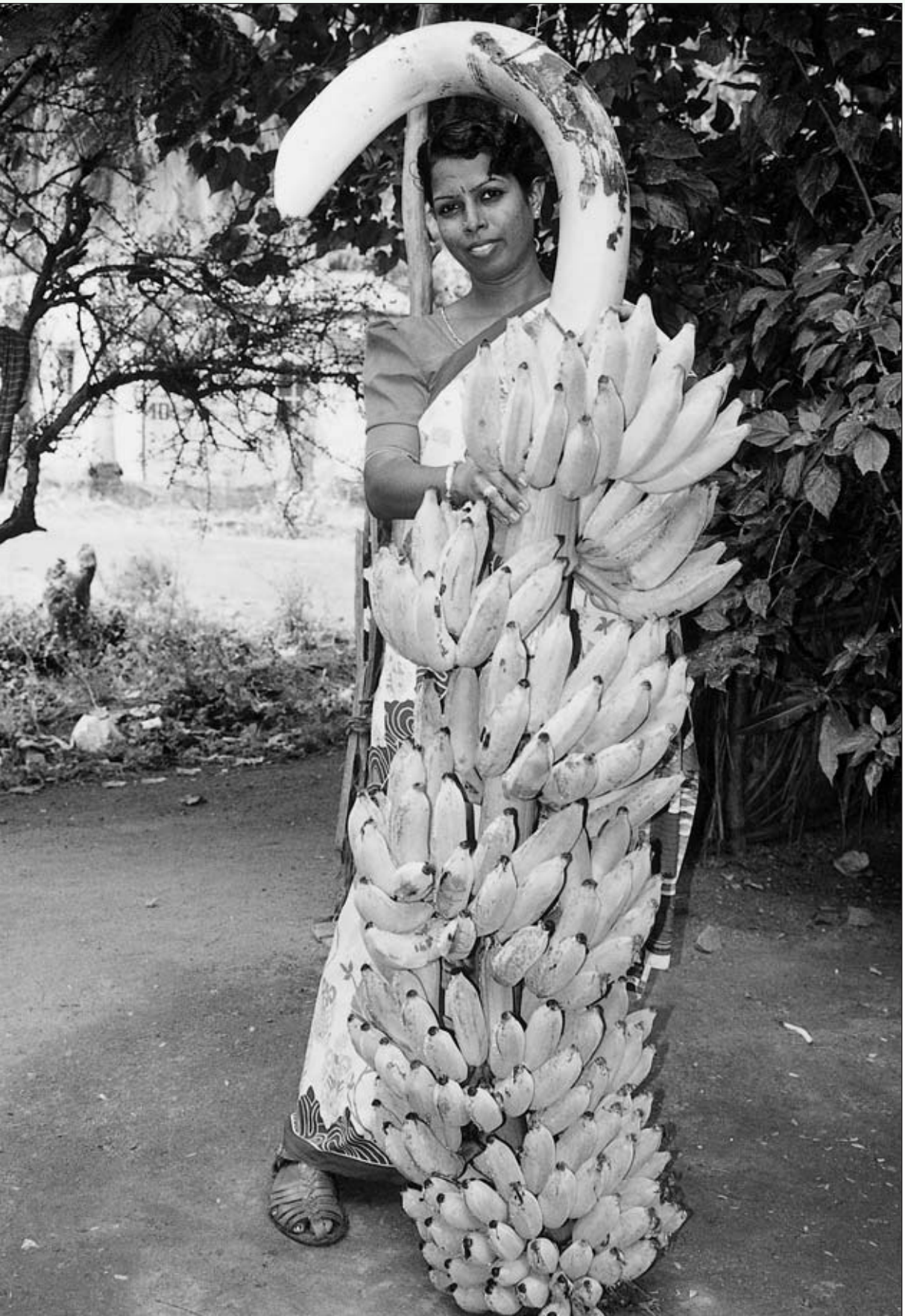
INFO*MUSA*

The International Magazine on Banana and Plantain



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Uma Subbarayan of NRCB with improved 'Pisang awak'. (Sathiamoorthy, NRCB, India)

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The mission of the International Network for the Improvement of Banana and Plantain is to sustainably increase the productivity of banana and plantain grown on smallholdings for domestic consumption and for local and export markets.

The Programme has four specific objectives:

- To organize and coordinate a global research effort on banana and plantain, aimed at the development, evaluation and dissemination of improved cultivars and at the conservation and use of *Musa* diversity
- To promote and strengthen collaboration and partnerships in banana-related research activities at the national, regional and global levels
- To strengthen the ability of NARS to conduct research and development activities on bananas and plantains
- To coordinate, facilitate and support the production, collection and exchange of information and documentation related to banana and plantain.

INIBAP is a programme of the International Plant Genetic Resources Institute (IPGRI), a Future Harvest centre.

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A new look for INFO*MUSA*


INFOMUSA

The International Magazine on Banana and Plantain

For the first time in its history INFO*MUSA* is undergoing a makeover in response to the growing number of submissions we receive every year. Starting with the next issue, the layout will be different to accommodate a greater number of shorter articles.

Our goal is to give you, the readers, a better magazine, nicer to look at, but also more informative for everybody. Clearly and simply written manuscripts would help make the articles published in INFO*MUSA* accessible to the greatest number of readers, whether they are geneticists or economists. We, in turn, will provide short summaries for non specialists on noteworthy topics and open up some of our pages to editorials and debates.

Some sections will disappear from the magazine but the audience they target will still be kept abreast of the latest developments, only in another form. And just as INFO*MUSA* is undergoing changes, PRO*MUSA* will no longer be inserted in the magazine and will only be available in electronic form.

Having more articles in the same space will put pressure on future authors to be brief and to the point. An article, illustrations included, should fit within 2½ pages of the magazine. Authors should aim for 2500 words, including references. The length of the text will vary depending on the number and size of tables and figures.

This adjustment can be done without jeopardizing the quality of articles. As you most certainly noticed, many articles begin with an introduction on bananas. Leaving them in the past probably encouraged emulation, but if there is a public that does need not to be educated on the importance of bananas as a staple food or a traded commodity, it is the readership of INFO*MUSA*. From now on, the introduction should only be used to provide the rationale for the research and any relevant background information.

'Materials and methods' is another section that could often be shorter. The authors should provide enough details of their experimental design to allow the reader to gauge the validity of the research. Similarly, only the tables and figures necessary for the comprehension of the article should be included. The information contained in small tables and histograms, for example, can generally be stated briefly in the text instead.

If possible, unpublished reports and not widely circulated documents, such as annual reports, should be avoided in the reference section. Our 'Instructions to authors', at the end of the magazine, explains how references should be cited. We are aware of being somewhat unorthodox on that front, so we ask future authors to pay attention to our guidelines and follow them when preparing a manuscript for INFO*MUSA*.

Just as idiosyncratic is the nomenclature of banana cultivars. Everybody is aware of the need to standardize the numerous ways of writing a cultivar's name. Until such a reference list becomes available, we ask authors to use single quotation marks, to capitalize the first letter of the name, but not the second part if there is one, and to avoid as much as possible local variations or translations, such as 'Gran Enano' instead of 'Grande naine'.

Vernacular names for parts of the banana plant should also be avoided. INIBAP has produced a small booklet, 'Descriptors for bananas' which can be consulted. It is available on INIBAP's website, like the thesaurus which is also trilingual.

We hope you will enjoy the new INFO*MUSA* we are preparing for you and keep sending the articles that are its 'raison d'être'. Feel free to write to us, whether it is in the form of a short letter or a commentary. We are always eager to hear from you and will do our best to publish your views, and to do it on schedule. You probably also noticed a discrepancy between the date on the cover of INFO*MUSA* and the date you received it. We apologize for our lateness and for any delays we have taken in communicating with the authors about the fate of their articles or other matters. From now on we will try to be more responsive to your needs and requests, and to keep you informed in a timely manner.

The editors

Comparative study of variability produced by induced mutation and tissue culture in banana (*Musa sp.*) cv. 'Grande naine'

L. R. Garcia, P.J. Pérez, I.C. Bermúdez, P.P. Orellana, N.R. Veitía, Y.M. Padrón and C.Q. Romero

Because the majority of plantain and banana clones are triploid, sterile and produce parthenocarpic fruits, improvement by crossing is extremely difficult. Therefore improvement by mutation and biotechnology is increasingly used for this crop (Donini and Sonnino 1998, Nichterlein 2000).

When Larkin and Scowcroft (1981) postulated the principle of somaclonal variation it raised considerable expectations and many laboratories started improvement programmes using tissue culture. Several years of work later, however, expectations have yet to be fulfilled since this technique is not very efficient at improving specific characters (Vuylsteke 2001).

Systems of improvement that use induced mutation based on mutagenic agents produce changes similar to natural mutations, but in a relatively shorter time and in greater quantity (Donini and Sonnino 1998). In *Musa*, chimaeras result from mutagenic treatment of multicellular structures and diplontic selection is a major obstacle to the isolation of induced somatic mutants.

Several researchers suggest that mutations produced by somaclonal variation are similar to those produced spontaneously or by mutagens (Pérez 1998b). There are no reports of studies with the cultivar 'Grande naine' that relate the variability in plants obtained during tissue culture to the variability obtained from a combination of nuclear techniques and tissue culture.

The purpose of this study was to compare the phenotypic variation of plants regenerated from adventitious buds which had been treated with ^{60}Co gamma radiation, with plants regenerated from adventitious buds which had not been exposed to radiation.

Materials and methods

The starting materials were shoot tips of 'Grande naine' obtained from sword suckers approximately 50-100 cm in height that were established *in vitro* in accordance with the methodology proposed by Orellana (1994). Adventitious buds were induced in accordance with the protocol proposed by García (2001).

Explants of 1 mm^2 derived from adventitious buds were treated with 25 Gy gamma radiation from a ^{60}Co source of 1.98 Gy/min. Immediately after treatment, the explants were transferred to fresh culture medium to avoid any toxicity that may have been produced by the action of the radiation on the constituents of the culture medium. Six thousand plants were regenerated from tissues treated with the mutagenic agent and 5000 from adventitious buds not exposed to radiation with both sets being manipulated the same way *in vitro* and *ex vitro*. As a control, 100 suckers obtained from corms were planted at random.

Rooting was induced using the methods proposed by Orellana (1994). Acclimatization was according to the technical instructions for the micropropagation of plantain (MINAGRI 1992).

Several characters such as plant height (cm), circumference (cm) and colour of the pseudostem, leaf and vein colour, colour of petiole, leaf habit and texture, appearance of bunch, type of rachis, form and colour of male buds were evaluated with reference to 'Descriptors for banana' (INIBAP 1996).

The proportion of variants, the percentage of each variation in relation to the total phenotypic variations observed, and the frequency of occurrence with respect to the total plants were determined. The data were analysed statistically by the chi-square test using the software package STATGRAFICS ver. 4.1 for Windows.

Knowing the importance of black leaf streak disease in banana and the resultant damage, the reaction of the plants to the disease was studied, treating each plant as an experimental unit and evaluating the disease at harvest.

The plantation was established in an area of the *Estación Experimental de Remedios* '*Pedro Lantigua*' which has a red ferrolytic soil (Jiménez *et al*. 1994). The distance between plants was 3.0 x 1.0 m and cultivation was in accordance with the instructions for the cultivation of plantain published by MINAGRI (1994).

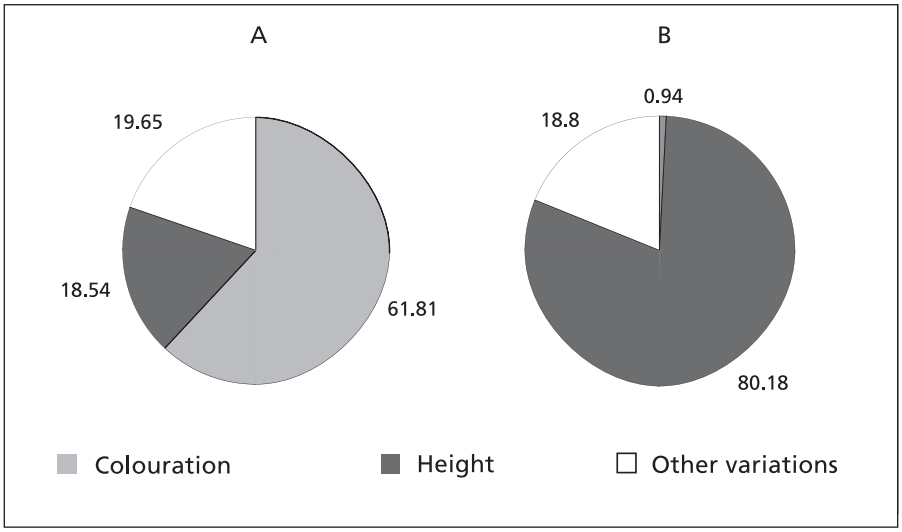

Figure 1. *Percentages of the main phenotypic variations in relation to the total variations obtained in: A) Population with mutagen treatment; B) Population without mutagen treatment.*

Results and discussion

Assessments of the two plant populations showed that phenotypic variations differed quantitatively and qualitatively, with a larger proportion of variants in tissue cultures with irradiation treatment (16.5%) in comparison with plants regenerated from non-radiated adventitious buds (6.6%).

Amongst the phenotypic variations found in plants obtained without mutagen treatment, the majority of variations were of reduced plant height (80.2% of total phenotypic variation), while variation in colour was 0.9% (Figure 1). In this population (no irradiation) there were other types of variation such as erect leaf habit,

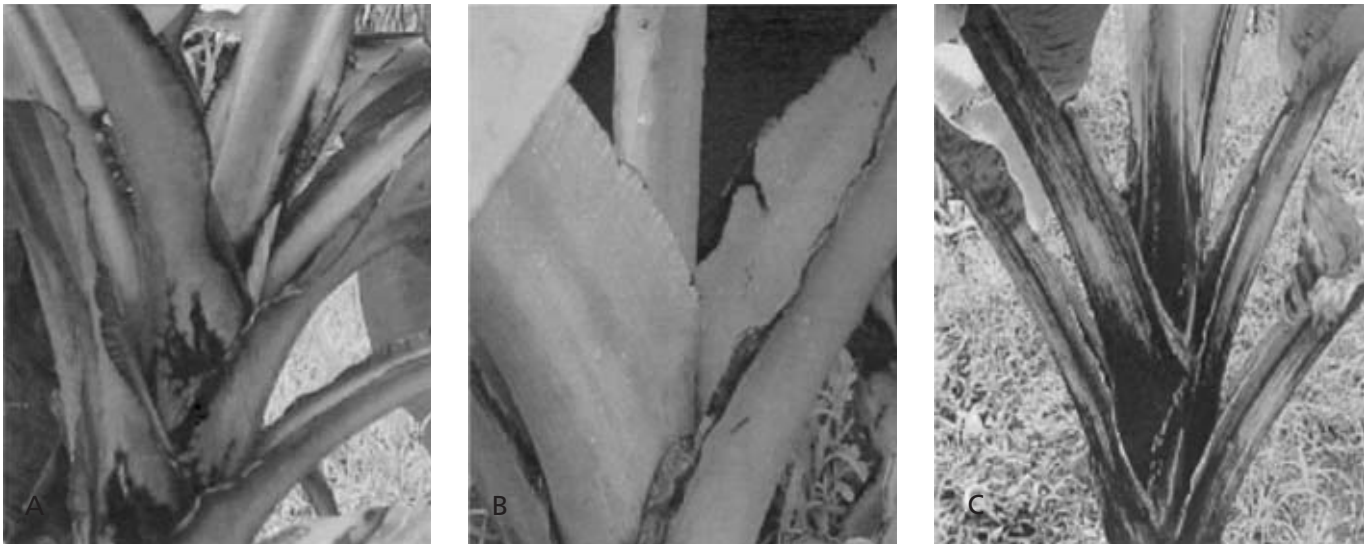

***Figure 2**. Various phenotypic variations found in the colour of plants obtained from irradiated adventitious buds: A-B) Change in colour to petiole margin; C) Abundant spots of dark greyish brown colour on the petioles and pseudostems.*

***Figure 3**. Various phenotypic variations in height of plants regenerated from irradiated adventitious buds : A) Dwarf plant; B) Grele.*

thin pseudostems, plants of 'Valery' type, and changes in the inflorescence and bunch, which accounted for 0.6, 0.6, 13.8 and 3.8% of the total observed phenotypic variation respectively.

The majority (61.2%) of variations in the population obtained by induced mutation were about colour, with variations causing reduced height accounting for only 18.5% of the total variations observed (Figure 1). In this population (with irradiation) there was a wide range of phenotypic variation as shown in figures 2, 3 and 4. Some sorts of phenotypic variations were not observed in plants obtained without mutagen treatment, e.g. distortions in the form and texture of the leaves (coriaceous or leathery), and early flowering (Table 1). Changes in pseudostem colour, a dark greyish brown colour of the edge of the petiole, and whitish stripes on the leaves and pseudostem were also not seen in plants regenerated from buds which had not been irradiated, where the only variations were in the shade of the reddish colouration.

Table 1. Frequency of occurrence of phenotypic variation in populations with or without mutagen treatment (gamma radiation) in cv. 'Grande naine'.

Phenotypic variation	Frequency (%)	
	Population with mutagen treatment	Population without mutagen treatment
Reduced height	3.49 b	5.44 a
Leathery texture	0.46	-
Changed colour	11.61 b	0.06 b
Leaf habit	2.24 a	0.042 b
'Valery'	0.48 b	0.93 a
Whitish strips on leaves and pseudostem	0.11	-
Early flowering	0.26	-
Inflorescence and bunches	0.24 a	0.25 a
Differential response to black Sigatoka	0.018	-

Values with different letters in the column differ at P<0.05, according to the chi-square test.

In the assessments of the inflorescences and bunches, some variations that were observed in plants obtained with mutagen treatment, such as reversion of the bunch from 'French' to 'Horn' types, naked rachis and changes in coloration of the male bud from purple to yellow, were also not found in the population obtained from adventitious buds without radiation (Figure 4). In the plantation originating from irradiated buds one plant showed a difference in reaction to black leaf streak disease, and was selected. All the plants obtained from adventitious buds without irradiation proved to be highly susceptible. Studies are continuing to determine the stability of the differential reaction to the disease.

Table 1 shows the main phenotypic variations found in each population. It is clear that changes in plant colour and leaf habit were dependent on the source of variability used, there being a much higher frequency of these characteristics in the population obtained by irradiation. According to the chi-square test, the frequency of plants with reduced height was more dependent on the source of variability than that of the 'Valery' type variation. In contrast, the frequency of occurrence of changes in the inflorescence and bunch were not dependent on the source of variability.

Somaclonal variation has been reported in the banana subgroup 'Cavendish' produced by micropropagation (Israeli *et al.* 1991) but the variation was always associated with problems in the size and quality

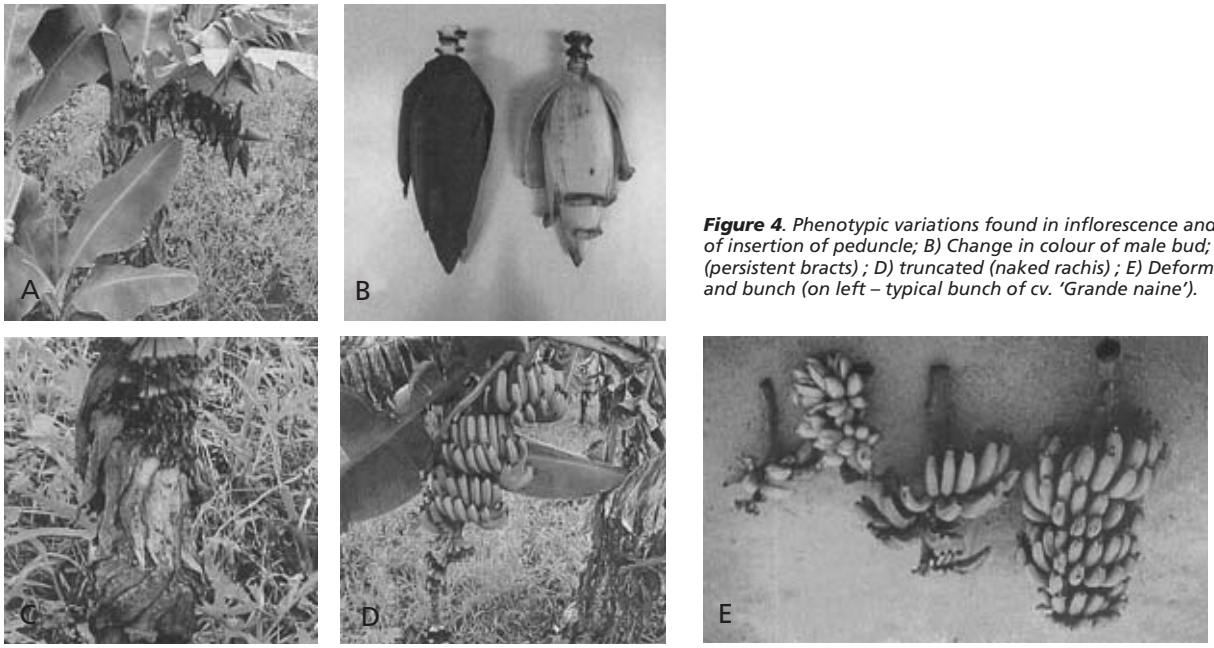

***Figure 4**. Phenotypic variations found in inflorescence and bunch: A) Angle of insertion of peduncle; B) Change in colour of male bud; C) Type of rachis (persistent bracts) ; D) truncated (naked rachis) ; E) Deformation of fingers and bunch (on left – typical bunch of cv. 'Grande naine').*

of the fruit (Vuylsteke *et al.* 1996). Vuylsteke (2001) indicated that somaclonal variation should not be overestimated as a source of variation for the genetic improvement of banana.

The majority of cultivars of plantain and banana originated by spontaneous mutation. According to the FAO/IAEA database, two banana cultivars obtained by induced mutation have been released (Nichterlein 2000).

The results of the present study are in agreement with those proposed by Ahloowalia (1998) who observed that variations in plants of potato (*Solanum tuberosum* L.) regenerated by tissue culture, and those obtained from treatment with a mutagen, differed completely with respect to the frequency and range of variation. Similar results were obtained by Schum and Preil (1998) in *Chrysanthemum.*

In a comparison of somaclonal variations and induced mutations in sugar cane, Pérez (1998b) found that the variability produced only by cultivation *in vitro* did not give the results that were hoped and it was the use of mutagenesis *in vitro* that was very effective.

All that has been shown above demonstrates that the highest rate and range of phenotypic variation was obtained when the adventitious buds were treated with gamma radiation, resulting in variations of interest to agriculture such as plants with early flowering and with a differential response to black Sigatoka. In the irradiated population, 68% of variations were distinct and not produced in plants regenerated from adventitious buds which had not been irradiated. Estimation of the variation is able to provide a measure of the possibilities of selection in populations (Pérez 1998a). The results obtained indicate that these possibilities would be more numerous in a population regenerated from irradiated buds, than in a population obtained by tissue culture alone. The variability of the character under improvement and the frequency of mutation of the gene(s) involved in this character will determine the size of the population needed for a successful outcome. ■

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Study of experimentally-induced variants of 'Manzano' (AAB) and 'Gros Michel' (AAA) bananas for their potential resistance to Fusarium wilt

I. Bermúdez, L. Herrera I., P. Orellana, N. Veitía, C. Romero, J. Clavelo, L. García, M. Acosta, L. García and Y. Padrón*

The oldest reports of Fusarium wilt, also known as Panama disease and caused by *Fusarium oxysporum* f.sp. *cubense* E. F. Smith (*Foc*), come from Cuba, Puerto Rico and Jamaica. According to Stover (1962), in Cuba the disease mostly affected the clones 'Gros Michel' and 'Manzano' (subgroup Silk). Since then, 'Gros Michel' and 'Manzano' have been increasingly affected by the disease and have disappeared from the market. Together with Sigatoka disease, also known as yellow Sigatoka, Fusarium wilt is responsible for the collapse of the banana industry in the 1940s (Battle and Pérez 1999). It is for this reason that in recent years the search for different sources of resistance to this devastating disease has intensified.

The combination of improvement by mutation and *in vitro* culture has made the induction and selection of somatic mutations more efficient. I*n vitro* culture has been used as a system for the induction of mutations in various *Musa* genotypes with different ploidy levels and combinations of the genomes of *Musa acuminata* (A) and *M. balbisiana* (B) (Novak *et al.* 1986*)*.

Following this approach of using biotechnological methods for genetic improvement, this report describes the results of investigations into the application of a selection methodology by means of inoculation with the pathogen, established by the authors, in order to evaluate the agronomic characters of potentially resistant or tolerant to Fusarium wilt variants obtained by exposing two susceptible cultivars to physical mutagens.

Materials and methods

The general procedures for obtaining vegetative material were as described by Orellana *et al.* (1991). The explants for the induction of adventitious buds were transferred to Murashige and Skoog medium supplemented with 20 mg/L of 6-benzylaminopurine and 0.65 mg/L indoleacetic acid (IAA). Adventitious buds were irradiated with 25 Gy gamma radiation from a ^{60}Co source. The resultant 5000 *in vitro* plantlets of each cultivar were inoculated for 30 minutes with a suspension of 3 x 10^5 spores/mL of strain INIFAT-1, a strain previously shown to have the highest pathogenicity. The plants were then transferred to earthenware pots containing an organic soil amended with the remains of plants with *Foc* disease. After 60 days, plants that were free of yellow foliage were re-inoculated with 1 mL of a suspension of spores of the same strain by puncturing the base of the pseudostem. Six months later, plants without symptoms were transferred to a field contaminated with the pathogen, for evaluation of disease incidence. Forty-two clonal lines were selected and multiplied *in vitro* to obtain 100 individuals of each. Each clonal line was then processed in the previously described manner until the pot stage. For further selection, the level of resistance was evaluated in pots and in the field according the following scale as proposed by Hwang (1991) and modified by the authors:

Scale	Range of infection (%)	Resistance level
1	0-10	Highly resistant (HR)
2	11-20	Resistant (R)
3	21-30	Moderately resistant (MR)
4	31-40	Moderately susceptible (MS)
5	41-50	Susceptible (S)
6	+50	Highly susceptible (HS)

Of the plants in pots that ranked 3 or less, 60 healthy plants were transferred to an experimental area of a typical red ferrolytic soil under natural conditions of infection, in order to evaluate agronomic characteristics in the presence of the disease at the field stage.

Data were subjected to a one-way analysis of variance. Duncan's multiple range test was used to determine homogeneous and/or significantly different groups, at $P<0.05$, previously checking the data for homogeneity of variance and normal distribution. Data that did not fulfil these conditions were subjected to Dunnet's non-parametric test of comparisons of means using the statistical package SPSS/PC v. 9.00 for Windows.

Results and discussion

The study resulted in 11 486 *in vitro* plants of both cultivars. These were subjected to rigorous selection procedures against the pathogen and showed different responses both in pots and in the field in the first and second cycles of selection. When inoculated and re-inoculated, 42 plants that were selected in the field gave different responses in the presence of the pathogen, expressed as percentage survival. The evaluation of 32 selected lines in a field with a natural infestation, resulted in a final selection of nine somaclones (Table 1).

As can be seen in Table 1, after the different evaluation procedures, clonal lines derived from 'Manzano' were discarded because they reached a level of infection over 30%, grade 4 of the evaluation scale proposed by Hwang (1991) and modified by the authors.

Of the plants in pots ranked 3 and under, 60 healthy plants were transplanted under conditions of natural infestation in order to evaluate their agronomic characteristics and their reaction to the disease.

The percentage of infected plants for the nine selected variants (0.17% of the original 5314 plants) of the cultivar 'Gros Michel' are shown in Table 2. The plants had levels of infection less than 30%; eight variants were at level 1 (HR) and one was at level 2 (R). The controls had levels of infection greater than 40%, which confirms the presence of the pathogen in the soil and the resistance of the selected variants.

In the last few years, the use of techniques to induce mutations in banana and plantain, has given rise to various variants resistant to Fusarium wilt (Hwang 1991, De Beer and Visser 1995), with increased bunch weight (Smith *et al.* 1994), reduced plant height (Bermúdez 2000) and early flowering (Roux *et al.* 1994).

Some variants of 'Gros Michel' (Tables 2, 3) stand out for their superior agronomic characters in comparison with the control:

- IBP 5-61 had a vegetative cycle that was shorter (15 months) than that of the donor cultivar (17 months). Other characteristics e.g. bunch weight and numbers of hands and fingers per bunch remained similar to 'Gros Michel' but this variant had a very uniform maturation of the bunch, and was highly resistant (3% infection).

Table 1. Results of selection against *Fusarium oxysporum* f.sp. *cubense*.

Clones	*In vitro* plants inoculated in Phase III and in plots	*In vitro* plants sown in the field	Material selected in the field	Material selected in plots	Variants selected in the field
'Manzano'	6172	934	20	11	-
'Gros Michel'	5314	213	22	21	9
Total	**11 486**	**1147**	**42**	**32**	**9**

- IBP 5-B had a vegetative cycle similar to that of the control 'Gros Michel', a good bunch weight, and increased numbers of fingers per bunch, which were also bigger. Of more importance, resistance was maintained in field conditions, with only 3.2% infection by the pathogen.
- IBP 12 had similar characteristics to the control in terms of bunch weight and numbers of hands per bunch, but developed larger numbers of fingers per bunch, which were also larger. As with IBP 5-B, infection by the pathogen in field conditions was 3.2%.
- The other variants had low levels of infection but yield and development cycle were not favourable. In particular, IBP 5-42 and IBP 5-6 had the lowest infection rates (Table 2) but also had two of the lowest bunch weights (Table 3).
- IBP 5-66, IBP 13-A, IBP 13-B and IBP 5-5 had premature shedding of the fingers during maturation, which is undesirable for production. It is important to emphasize that plants with high resistance do not necessarily have good agricultural characteristics or yield.

Similar results were obtained by Hwang and Ko (1988) who obtained variants that were highly resistant to *F. oxysporum*, but all were morphological variants with combinations of agronomic characters that were inferior to the original cultivar.

Conclusion

With this work, we showed the possibility of using biotechnological methods such as mutations to improve plantain and banana. At present, three variants derived from 'Gros Michel' have shown resistance to *F. oxysporum* and have good agricultural characteristics, a result that is of great practical value. ■

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Table 2. Percentage of infection of the variants selected for their resistance to *Fusarium oxysporum* f.sp. *cubense* in field conditions.

Code	% of diseased plants in the field	Resistance level*
IBP 5-42	0.2	HR
IBP 5-6	2.5	HR
IBP 5-61	3.0	HR
IBP 5-B	3.2	HR
IBP 12	3.2	HR
IBP 13-A	3.3	HR
IBP 5-66	4.0	HR
IBP 5-5	5.5	HR
IBP 13-B	12.5	R
'Manzano'	48.0	S
'Gros Michel'	43.0	S

*HR: highly resistant; R: resistant; S: susceptible

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Table 3. Comparison of agronomic characters of the variants derived from 'Gros Michel' that were resistant to *Fusarium oxysporum* f.sp. *cubense*.

Variants	Growth cycle (months)	Plant height (cm)	Pseudostem diameter (cm)	Bunch weight (kg)	Number of hands per bunch	Number of fingers per bunch
IBP 13-A	16.8	332.5 c	55.10 cd	14.02 ab	7.8 bcd	108.2 abc
IBP 13-B	16.5	343.4 bc	58.15 bcd	10.42 b	8.5 abc	108.4 abc
IBP 5-6	17.0	368.5 abc	62.30 abc	11.84 b	8.8 abc	119.6 a
IBP 5-5	18.0	370.6 ab	59.30 bcd	13.57 ab	9.0 ab	113.4 ab
IBP 5-66	18.0	387.5 a	63.40 ab	14.52 ab	9.3 a	117.2 ab
IBP 5-61	15.0	353.5 abc	54.5 cd	15.89 a	9.0 ab	116.4 ab
IBP 5-42	15.5	335.7 bc	53.40 d	10.88 b	7.5 d	100.0 c
IBP 5-B	17.0	343.3 bc	59.20 bcd	17.05 a	9.0 ab	120.5 a
IBP 12	17.0	336.0 bc	59.95 bcd	16.82 a	8.6 abc	114.0 ab
'Gros Michel'	17.0	359.0 abc	67.60 a	16.92 a	8.9 ab	103.5 bc
EE	-	±3.76	±0.85	±0.41	±0.13	±1.50

Means followed by different letters in the same column differ by the Duncan or Dunnett's tests at p<0.05.

Genetic transformation by *Agrobacterium tumefaciens* of embryogenic cell suspensions of plantain 'Dominico hartón' (*Musa* AAB Simmonds)

C.R. Pineda, N. Toro Perea, J. Narvaez, M.L. Orozco, A. Laignelet and H. Cárdenas*

Musa crops are attacked by various viruses, bacteria and fungi. Control is by means of cultural practices and powerful fumigation with chemical products that not only damage the environment and human health, but also increase production costs. Application of traditional methods of improvement have not given the expected results, because of the limited knowledge on the diversity and genetics of the pathogens, and the problems posed by the triploidy, low fertility and long generation times of bananas. Genetic engineering offers an alternative for overcoming these limitations, because of the possibility of introducing specific genetic changes in a short period of time (Sági *et al.* 1994).

There is a variety of methods for gene transformation of plant cells. Infection with the natural vector *Agrobacterium tumefaciens* is up to now the most promising, and better than particle bombardment and other gene transfer techniques, because of its simplicity and high efficiency of transformation, the possibility of transferring relatively large segments of DNA with defined ends and little rearrangement, and incorporation of a low number of copies in the plant chromosome (Gelvin and Liu 1994, Komari *et al.* 1996). Transformation methods with *Agrobacterium* are being applied successfully to dicotyledons and monocotyledons, and have been reported, for example, with cultivars of rice, maize, yucca and banana (Ganapathi 2001, Sagi *et al.* 2000). *Agrobacterium*-mediated transformation of meristems of *Musa* spp. var. 'Grande naine' has also been described (May *et al.* 1995).

This work determines the efficiency of transformation with *A. tumefaciens* of embryogenic cell suspensions of the plantain 'Dominico hartón' (AAB), based on the transient expression of the gene for ß-glucuronidase (GUS) and considers three factors: 1) concentration of *Agrobacterium*, 2) concentration of acetosyringone (AS) and 3) infection period. The results confirmed the potential of the method for transforming embryogenic cell suspensions with *A. tumefaciens* for the incorporation of foreign genes of interest in commercial varieties of plantain.

Materials and methods

Vegetative material and culture conditions

Embryogenic cell suspensions (ECS) of 'Dominico hartón' were established in the Laboratory for Tropical Crops at the *Katholieke Universiteit Leuven* (KULeuven), Belgium, with material derived from the *in vitro Musa* collection of INIBAP. ECS were maintained in MS liquid medium with half the macronutrients and with iron, 5 µM dichlorophenoxyacetic acid (2,4-D) and 1 µM zeatin as growth regulators (Dhed'a *et al.* 1991, Sági *et al.* 1995), at 26 ± 2°C, with continual shaking at 85 rpm, an illumination of 1000 lux and a photoperiod of 14/10 hours light/darkness. The ECS were homogenized, passed though a series of sieves of 1.0, 0.5, and 0.25 mm in diameter, and subcultured on the same medium for 4 days (Sági *et al.* 1995) at 80 rpm and 26 ± 2°C under fluorescent light.

Bacterial strain and plasmid

The ECS were infected with *A. tumefaciens* strain AT650, which contains the binary plasmid pLIGH, supplied by the *Centro Internacional de Agricultura Tropical* (CIAT). The vector is 9.5 kb, and is derived from pSG-ManI, which contains two chimeric genes: 1) the gene for the enzyme ß-glucuronidase of 2.5 kb with the *gusA*-intron, the 35S promoter of cauliflower mosaic virus (CaMV) and the CaMV 35S terminator; 2) the gene *hph* (hygromycin phosphotransferase) (31), of 1.7 kb that confers resistance to hygromycin with the CaMV 35S promoter and the tml (*tumour morphology large gen*) terminator.

Transformation

A. tumefaciens strain AT650/pLIGh was grown in semisolid AB medium (Gelvin and Liu 1994) with 300 mg/L streptomycin, 100 mg/L spectinomycin, and 50 mg/L hygromycin for 2 to 3 days at 28 ± 2ºC. Inoculum was prepared from cells of a recent culture in liquid AB medium with the same concentration of antibiotics, and was incubated for 12 to 16 h, with shaking at 28 ± 2ºC, until it reached a concentration of 1 to $2x10^9$ cells/mL. The culture was washed in sterile saline solution, and resuspended in two volumes of induction medium (Gelvin and Liu 1994) containing 2 mM sodium phosphate, 30 mM MES buffer at pH 5.6, sales solution AB 1X, 0.5% glucose, with 50, 100, 200 or 400 µM AS. The suspension was incubated at 25 ± 2ºC for 12 to 16 hours, and 100 rpm. The concentration of bacteria was adjusted to $1x10^8$ and $1x10^9$ cells/mL, by diluting with induction medium containing 50, 100, 200 or 400 µM AS. Culture medium for the ECS was replaced by the suspension of *Agrobacterium* at the appropriate concentration of $1x10^8$ or $1x10^9$ cells/mL. Infection was at 25 ± 2ºC, 140 rpm in darkness for 1, 4, 8, 12, 16, 20 or 24 hours. After infection, the ECS were washed in MS medium to remove excess bacteria. During the days following co-cultivation in the dark, MS liquid medium was used at half strength with 5 µM 2,4-D and 1 µM zeatin at pH 5.8 (Sági *et al.* 1995) with 50, 100, 200 or 400 µM AS. Finally, the medium was replaced by an equal volume without AS, and containing 250 g/L cefotaxime and 20 mg/L hygromycin.

Evaluation of transient GUS activity

Histochemical colorimetric analysis was according to the method of Jefferson *et al.* 1987. Tissue was fixed in a 0.27% formaldehyde solution, 0.01 mM MES and 5.46% manitol, submitted to a vacuum, washed three times with sodium phosphate (50 mM, pH 5.0) solution, and incubated overnight at 37°C in sodium phosphate buffer (50 mM, pH 7.0) containing 1 mM 5-bromo-4-chloro-3-indolyl-b-D-glucuronide (X-Gluc-GibcoBRL). After incubation, the tissue (ECS) was bleached with 2.5% sodium hypochlorite and examined under the stereomicroscope. The presence of aggregates of embryogenic cells with GUS activity (blue spots) was evaluated every 24 hours for 7 consecutive days after infection with *Agrobacterium*, and finally on the tenth day, for each treatment and replicate; data were expressed as percentages.

Experimental design and statistical analysis

The ECS of 'Dominico hartón' were infected with *Agrobacterium* in different conditions. Treatments corresponded to combinations of the factors that were analyzed, *Agrobacterium* concentration, AS concentration and infection period, as described in Table 1. The factorial arrangement was 2x4x7, with three replicates. Transient expression of *gusA* is presented as the percentage of cell aggregates with GUS activity for the first seven days, and for the tenth day after infection.

The experimental data were subjected to an analysis of variance and Tukey's multiple comparison test for the main factors and interactions. The components of variance were estimated by means of the statistical package SAS, version 6.3. Means and confidence limits of the main factors and interactions were prepared graphically with the programme *Stat Graphics*.

Table 1. Factors and treatments tested in the transformation of embryogenic cell suspensions of 'Dominico hartón' by *Agrobacterium tumefaciens*.

Factors	Levels	Treatments
a: Concentration of	a1: $1x10^8$ cells/mL	
Agrobacterium	a2: $1x10^9$cells/mL	a1b1c1 to a1b1c7
b: Concentration of	b1: 50 µM	a1b2c1 to a1b2c7
Acetosyringone	b2: 100 µM	a1b3c1 to a1b3c7
	b3: 200 µM	a1b4c1 to a1b4c7
	b4: 400 µM	
c: Infection period of ECS	c1: 1 h	a2b1c1 to a2b1c7
	c2: 4 h	a2b2c1 to a2b2c7
	c3: 8 h	a2b3c1 to a2b3c7
	c4: 12 h	a2b4c1 to a2b4c7
	c5: 16 h	
	c6: 20 h	
	c7: 24 h	

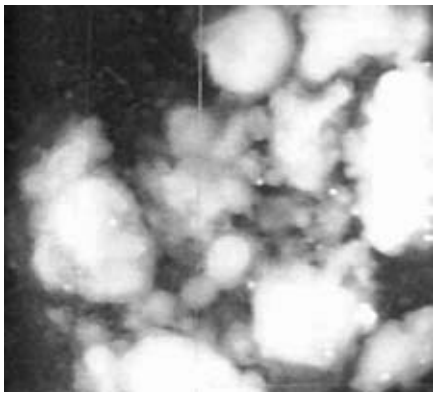
Results

GUS activity in ECS of 'Dominico hartón'

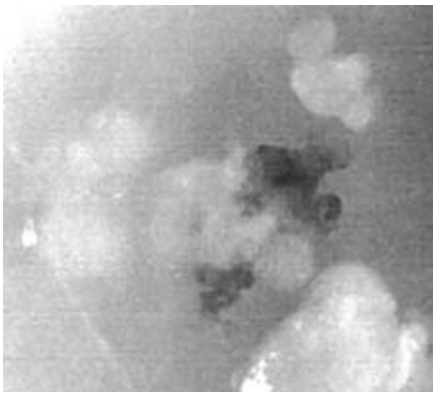
GUS activity in ECS of 'Dominico hartón' infected with At650/pLIGh was shown by histochemical analysis (Figure 1). Since the *gusA* gene in the LIGH plasmid contains an intron that blocks its expression in bacterial cells, it is expressed only in transformed plant cells. As non-transformed plant tissue has no endogenous expression, it was concluded that the GUS activity observed in the ECS was due to expression of the reporter gene in the transformed embryogenic cells (Figure 1).

Interactions between the factors that were analyzed made a large contribution to the transformation process

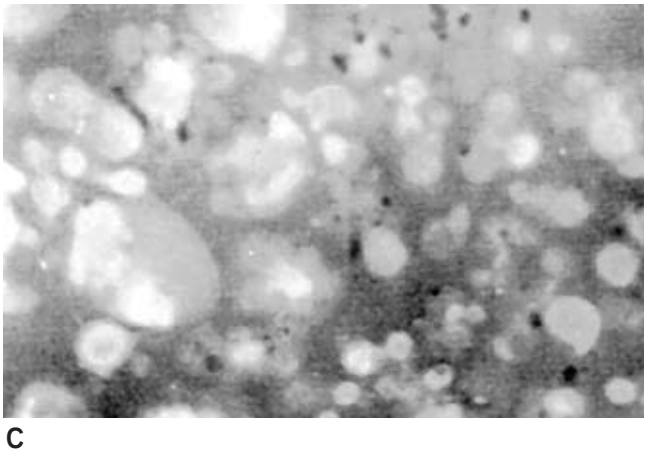
Statistical analysis of the interactions axb, axc, bxc and axbxc show their importance to the transformation process ($P<0001$), and that the largest average efficiency of transient expression was produced by the interactions a1xb3 (34.96%), a1xc7 (32.76%), b3xc7 (41.21%) and a1xb3xc7 (61.10%).



A



B



C

Figure 1.
Histochemical coloration of embryogenic cell suspensions of 'Dominico hartón' transformed with At650/pLIGh showing GUS activity. Cell aggregates in MS medium at half the concentration with 5 µM 2,4-D and 1 µM zeatin, stained histochemically, 72 h after infection: A) no infection; B) and C) infected for 24 h with $1x10^8$ cells/mL of At650/pLIGh, in the presence of 200 µM AS.

Estimation of the components of variance suggests that the interaction *Agrobacterium* x AS is the most important, with a contribution of 12.97% of total variance (Figure 2). In Figure 3-A, it can be seen that for both concentrations of *Agrobacterium*, the highest efficiency of transformation was with 200 µM AS, and that $1x10^8$ cells/mL At650/pLIGh gave the highest number of cell aggregates having GUS activity in comparison with the other concentration, at the four levels of AS tested.

The interaction AS x infection period confirmed that 200 µM AS was the concentration that showed the highest efficiency for the interval 12-24 h of infection (Figure 3-B).

The interaction infection period x *Agrobacterium* contributed the least to the total variance (6.39%) (Figure 2). Figure 3-C confirmed the superiority of $1x10^8$ cells/mL as regards the efficiency of transient expression for all periods of infection.

For the interaction between the three factors, the contribution of the average variance to the total variance was 8.2% (Figure 2), which shows that although important it is not the most important of the interactions analyzed.

Transient expression for the days of evaluation

Although the infection period was not considered to be a factor of major importance in the transformation process, because the results did not depend on the main factors studied, the interaction of infection period with the time of evaluation provided information on the variation in the levels of efficiency for transient expression during the 10 day evaluation. Figure 4 shows that the highest frequency of transformation was observed after three days for a 24 h infection period (37.62%), but by the tenth day it had fallen to 10.57%. Also, it was

observed that for the 1, 4, and 8 h infection periods, GUS activity became evident from the second day of co-cultivation (0.44 to 2.12%), but was zero by the tenth day. The opposite occurred for the 12, 16, 20 and 24 h infection periods, where the action of the enzymes was observed from the first day of co-cultivation (6.92 to 20.62%), and remained until the tenth day (3.88 to 15.35%). The highest efficiency of transformation by the tenth day was observed for the 20 h infection period (15.35%) (Figure 4).

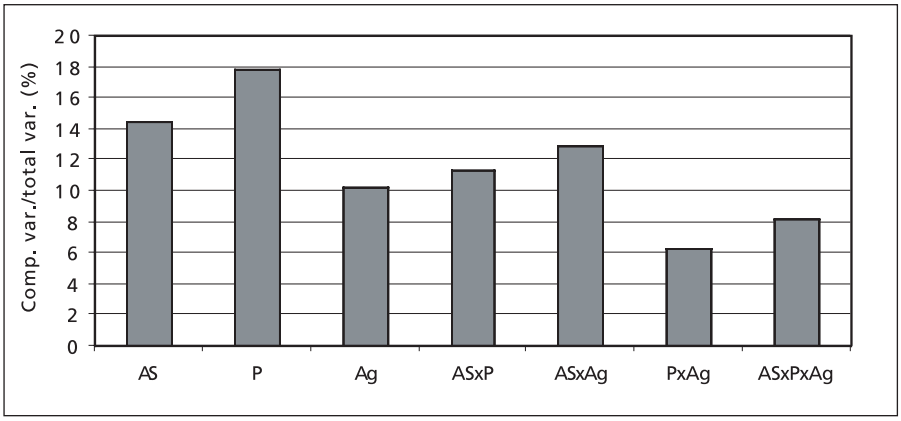


Figure 2. *Estimation of the components of variance for concentration of* Agrobacterium *(Ag), concentration of acetosyringone (AS) and infection period (P); and interactions between them.*

Discussion

In this study we obtained transient expression of the *gusA* gene in ECS of 'Dominico hartón' after *Agrobacterium*-mediated transformation, as shown by GUS activity in cell aggregates when evaluated by histochemical analysis. The use of the *gusA*-intron, confirmed that the GUS activity was due to the transfer of T-DNA in the plant cell and not to expression in the cells of *Agrobacterium*.

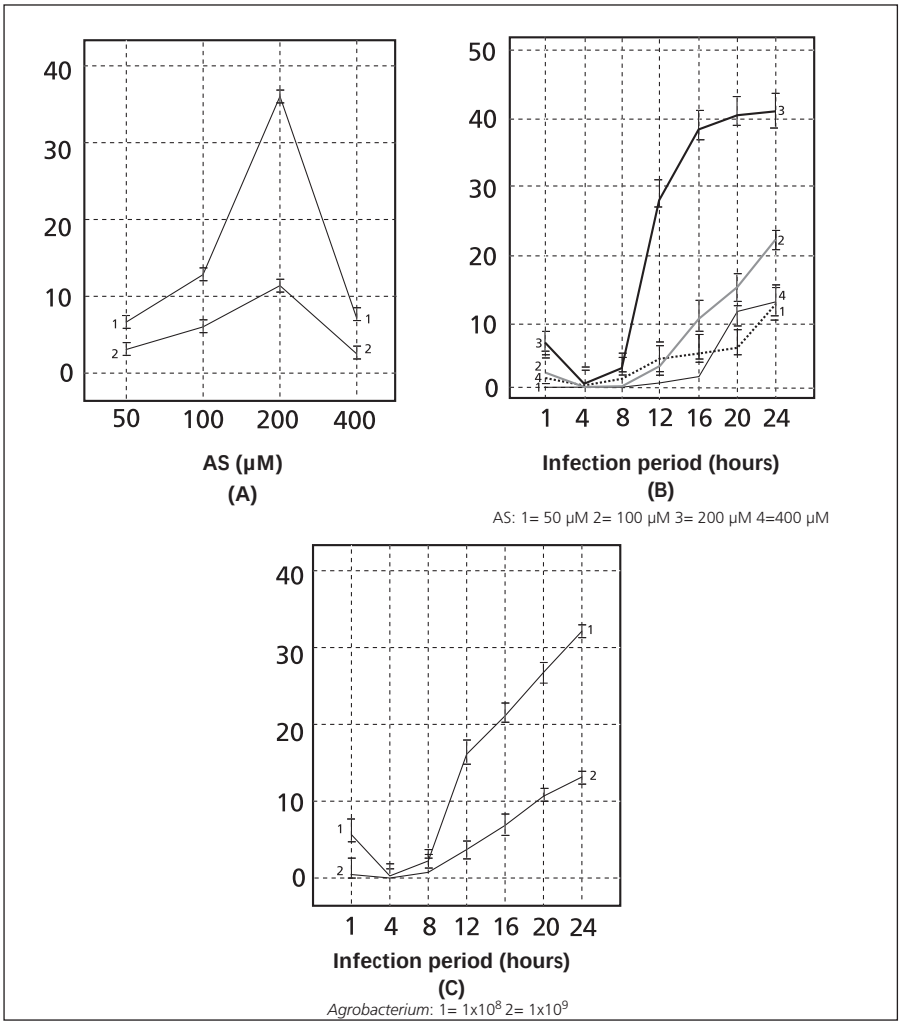
Before starting the transformation experiments, the sensitivity of 'Dominico hartón' ECS to the antibiotics used after infection was tested. A dose of 250 mg/L cefotaxime, selected to inhibit growth of *Agrobacterium*, had no effect on the regeneration of embryogenic cells.

Strain AT650 was effective for the transient transfer of T-DNA to 'Dominico hartón' ECS probably because it contained an inactivated Ti plasmid of a hypervirulent agropine type pTiBo542, which has proved effective with difficult plant species (Gelvin and Liu 1994, Hiei *et al.* 1994, Narasimhulu *et al.* 1996). The characteristics of the binary vector that was used is also very important when one is trying to achieve high transformation efficiency. The plasmid pLIGh used here has the *gusA* gene under the regulation of the CaMV 35S promoter which is efficient in other monocotyledons and has nopaline-type borders, which are more effective than octopine-type borders in monocotyledons such as maize (Shen *et al.* 1993).

The most efficient concentration of *A. tumefaciens* [$1x10^8$ cells/mL (15.04%)] coincides with the concentration used for maize (Ishida *et al.* 1996), but differs from other reports where the concentration was up to $1\text{-}2x10^{10}$ cells/mL (Shen *et al.* 1993). This may be explained by the type of tissue and the species of plant being infected. It is possible that the tissue was rapidly wounded at bacterial concentrations higher than $1x10^9$ cells/mL, or that the tissue was more susceptible to infection. Therefore a concentration of $1x10^8$ cells/mL should be sufficient for transfer of T-DNA and create less competition between cells for the nutrients in the culture medium.

It is known that AS increases transformation efficiency in dicotyledons as well as in wounded monocotyledons (Shen *et al.* 1993, Stachel *et al.* 1985, Sheikholesman and Weeks 1987, Delmotte *et al.* 1991, Hansen *et al.* 1994). The absence of GUS activity in treatments without AS demonstrates that the presence of this phenolic compound is essential for transformation. The absence may have been because the plant tissue had not been wounded, and by an inherent inability of monocotyledons to activate virulence genes. Although phenolic compounds induce *vir* genes in wounded monocotyledon tissues (Usami *et al.* 1988), they may not be sufficient for activation (Schäfer *et al.* 1987), therefore it may be necessary to add synthetic AS to the medium for induction, infection and co-cultivation.

Figure 3. *Mean and confidence limits of GUS activity observed for interactions between the factors analyzed. A:* Agrobacterium *x AS. B: AS x infection period. C:* Agrobacterium *x infection period.*



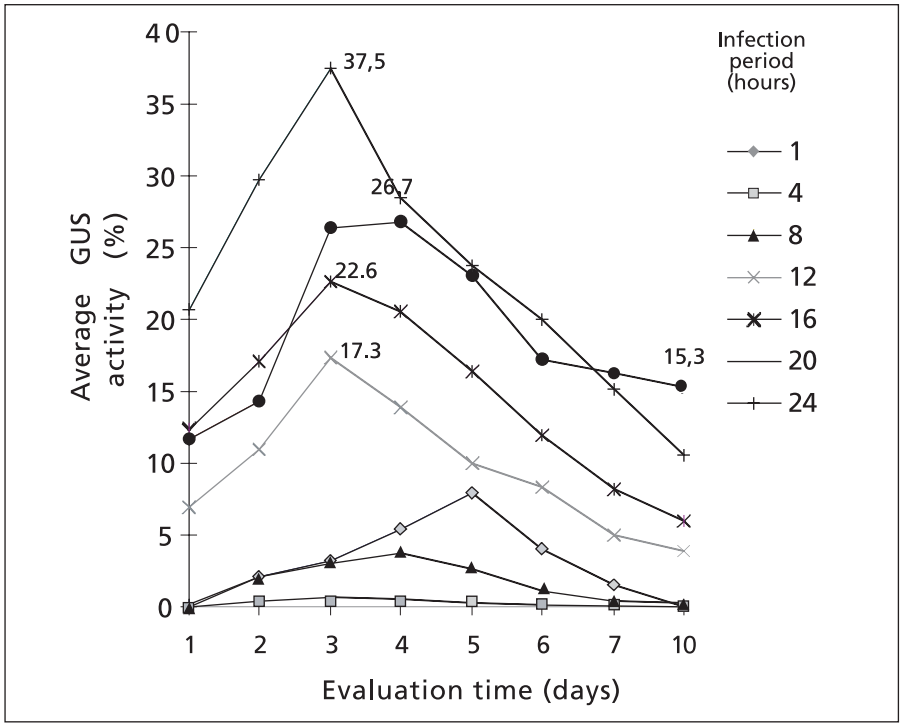


Figure 4. *Mean and confidence limits for the efficiency of transformation for the tested infection periods and evaluation times.*

Analysis of the independent factors shows that the best efficiency of transient expression was obtained with 24 h (23.24%). The infection periods that were studied did not yield the most efficient, but it is possible to say that for ECS the infection period must be between 16 and 24 h. It remains to determine whether a period of more than 24 h increases the rate of transformation or whether, on the contrary, the viability of plant tissue is reduced. These results show that transformation efficiency depends mainly on the infection period. The use of such a wide range (1-24 h) is because this factor is one of the most variable and depends on the type of plant tissue.

The study of the interaction between the three factors studied shows the importance of the combination of these factors for improving transformation efficiency.

Expression of the reporter gene was studied for the first days after infection. The interaction of infection period with the time of evaluation showed that the maximum peak of GUS activity occurred on the third day, and from then on declined. Possibly this is because of the action of the selective antibiotic on the ECS, added on the fourth day, indicating that the initial expression of the *gusA* gene was in most cases transient. The detection of GUS activity on the tenth day shows the possibility of obtaining stable transformants for several reasons: 1) transient expression of a gene is only seen in the first 4 to 6 days of transformation, returning to zero by the 10th day (Castle and Morris 1994); 2) from the 4th day, the selective antibiotic was active, and the GUS activity would only be detected in the transformed ECS (resistant to antibiotic) (Zheng *et al.* 1991); 3) *Agrobacterium*-mediated transformation offers a greater probability of incorporation of heterologous DNA within the plant genome, with the incorporation of small numbers of copies of the introduced genes (Hiei *et al.* 1994), and for the transfer of relatively large segments of DNA, with defined ends and with few rearrangements (Gelvin and Liu 1994, Hiei *et al.* 1994, Pineda and Orozco 1996, Ishida *et al.* 1996, Komari *et al.* 1996).

Conclusion

The possibility of obtaining stable transformants is supported by the stable expression obtained following infection with *A. tumefaciens* achieved in meristematic cells of banana 'Grande naine' (May *et al.* 1995). The efficiency of this methodology to achieve stable transformation is even more likely with embryogenic cell suspensions of *Musa* (Ganapathi 2001). Embryogenic cultures are an advantage because plants can be regenerated from a single cell, avoiding the formation of chimeric transformants. Also, the rate of multiplication is at least five times greater in comparison with the proliferation of cultured meristems (Dhed'a *et al.* 1991, Escalant and Teisson 1989, Panis *et al.* 1993). Furthermore, the use of actively dividing cells, such as embryogenic cells, favours the incorporation of T-DNA in the nuclear genome, by ensuring the presence of the enzymatic machinery needed for incorporation (Shen *et al.* 1993).

Using the present method for transformation of plantain 'Dominico hartón', based on the use of embryonic cell suspensions in their growth phase at a size of 0.25-0.5 mm, it is possible to achieve a transient expression of up to 37.5%, and it is probable that transgenic plants with a stable expression of the incorporated gene can be obtained.

Acknowledgements

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Genotypes of banana (*Musa* spp.) under saline stress: tolerance and sensitivity

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Production of bananas is limited in areas that have soils which are saline or contain excess sodium. These problems are caused by irrigation with saline water or result from inadequate soil management, and they are increasingly serious in arid and semi-arid regions of the world.

Soil salinity reduces crop growth and productivity because of the reduced osmotic pressure in the soil and the increase in certain ions to concentrations that are toxic to plants. These factors interfere in physiological processes such as transpiration, photosynthesis, translocation and respiration as well as causing imbalances in the water and/or ionic equilibrium of the plant (Richards 1992, Bohra *et al.* 1993).

Methods to restore saline soils are generally slow and expensive. The use of cultivars of banana which tolerate saline stress may provide a solution which is technically and economically viable. The selection of banana genotypes in soils that are naturally saline is difficult because of the large spatial and temporal fluctuations in the ionic concentration of soil. In order to avoid such natural variation at the first stage of selection, it is possible to use nutrient solutions with added salinity to control ionic concentration (Rawson *et al.* 1998).

The objectives of this work were: to evaluate the saline tolerance of five banana genotypes under greenhouse conditions, to study the effects of saline stress on physiological parameters, and to characterize the genetic diversity of the five genotypes by means of isoenzyme markers.

Materials and methods

Experiments were carried out in a greenhouse at the *Universidade Federal Rural de Pernambuco*, Recife, Brazil. The banana genotypes under evaluation were: 'Calcutta 4' (AA); 'Pacovan' (AAB, subgroup Prata), a mutant of the common Prata (Pome); 'Nanicão' (AAA, subgroup Cavendish); 'Caipira' ('Yangambi km 5', AAA) and 'FHIA-18' (AAAB).

Plants were produced by micropropagation and were approximately 15 cm in length. Cultivation was in black polythene bags (55 cm deep x 33 cm in diameter) that each contained 10 kg of sand. The surface of the substrate was covered with a 3 cm layer of gravel to reduce evaporation and to facilitate the control of

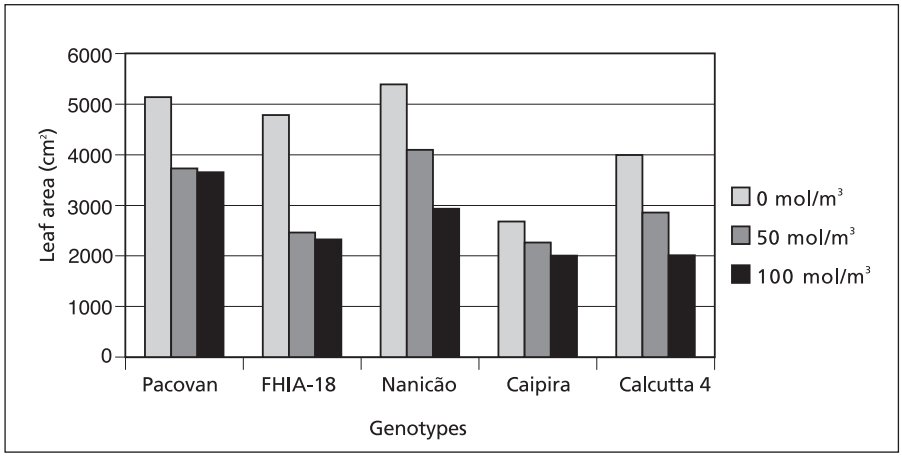


Figure 1. *Leaf area of five banana genotypes subjected to a 21-day saline treatment.*

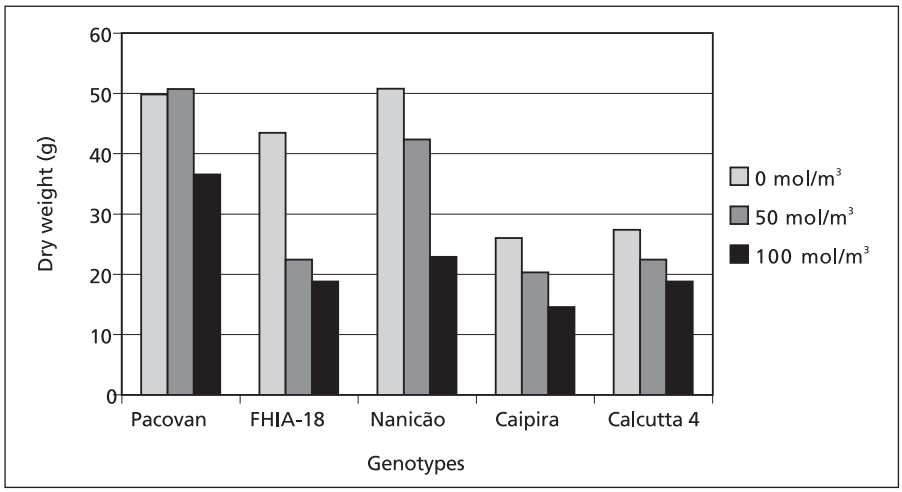


Figure 2. *Dry weight of five banana genotypes subjected to a 21-day saline treatment.*

salinity. Plants were drip irrigated daily for 15 min, at a rate of 0.6 L plant^{-1} day^{-1}. The irrigation solution contained 742.86 mg/L soluble fertiliser (Kristalon ®) with the following composition: 3% N, 11% P_2O_5, 38% K_2O, 4% MgO, 11% S plus micronutrients. In addition, 840 mg/L calcium nitrate (Barco Viking ®), equivalent to 15.5% N and 19% Ca, was applied. There were three treatments, prepared by the addition of NaCl to the nutrient solution and corresponding to 0, 50 and 100 M NaCl. The electrical conductivities of the solutions were 1.3, 6.5 and 11.7 dS/m respectively. Electrical conductivity was recorded every two days and adjusted as necessary.

The experiment was in the form of a completely randomized design, with three levels of salt and five cultivars (2 x 3 x 5), making a total of 30 subplots. Each subplot contained 4 plants of each genotype, with a total of 120 plants. Data were analyzed statistically by means of analysis of variance and comparison of means by the Tukey test at 5% probability. The parameters for evaluation were: leaf area, dry matter production, and salt concentrations of the vegetative tissue: sodium (Na^+), potassium (K^+), calcium (Ca^{2+}) and chloride (Cl^-). In addition, five isoenzyme systems were analyzed: peroxidase (PO), shikimic dehydrogenase (SKDH), malate dehydrogenase (MDH), glutamic oxaloacetic transaminase (GOT) and acid phosphatase (ACP).

At the end of the experiment, 21 days after the start of treatments, leaf area was estimated by multiplying the product of leaf width and length (cm) by a factor of 0.7 (adapted from Moreira 1987). Three types of plant part were selected: leaf lamina, pseudostem and root + rhizome. Dry matter content was determined by oven drying at 65ºC to constant weight. Each of the plant parts was analyzed for Na^+, K^+, Ca^+ and Cl^- concentrations.

After drying, vegetative material was ground and a sample digested in nitroperchlorate for sodium, potassium and calcium analysis. Na^+ and K^+ concentrations were determined by flame spectrophotometry, and calcium concentration was determined by spectrophotometry and atomic absorption (Malavolta *et al*. 1989). Chloride was analyzed by titration with silver nitrate previously extracted in aqueous solution (Malavolta *et al*. 1989).

Isoenzymes were analysed using 350 mg of fresh material from the lamina of the third leaf of each plant. Samples were ground in a cooled pestle and mortar with 1 mL buffer (Scandalios 1969), 300 mg saccharose and 300 mg polyvinylpyrrolidone (PVP). Homogenates were centrifuged at 14 000 rpm and 4ºC for 10 min and 10 mL aliquots of supernatant transferred to gels. Migration was at 4ºC and a potential of 9.0 v/cm, until the leading edge reached 9 cm in the direction of the positive pole (anode). Immediately afterwards, the gels were stained and photographed. For the ACP, MDH and SKDH isoenzyme systems, gels were prepared with 8 mL lithium borate buffer at 0.2 M and pH 8.3, and 72 mL citrate buffer at 0.2 M and pH 8.3; for the wells, lithium borate buffer was used at 0.2 M and pH 8.3. Gels of the GOT isoenzyme system were prepared with sodium acetate buffer at 0.2 M and pH 5.0; gels of the PO system were prepared with Poulik buffer for gels, pH 8.0 (9.2 g Tris, 0.96 g citric acid plus distilled water made up to 1000 mL). Wells for the GOT and PO isoenzyme systems contained Poulik buffer for wells (18.54 g boric acid and 2 g sodium hydroxide and distilled water made up to 1000 mL). All gels were prepared at a concentration of 7% (0.28 g bisacrylamide, 5.32 g acrylamide, 0.08 mL temed (tetramethylethylenediamine), 0.8 mL 10% ammonium persulphate and specific buffer 80 mL). Gels were fixed in a solution of AYALA (methyl alcohol, glacial acetic acid and distilled water in proportions of 1:1:1 v/v) for 20 min.

Data obtained from isoenzyme analyses and band colouration were tabulated in binary form with presence (1) or absence (0) of bands. Genetic similarities between the genotypes were estimated according to Dice's coefficient (equivalent to the index of Nei and Li 1979) in the NTSYS pc-programme (*Numerical Taxonomy and Multivariate Analysis System, version 1.70, Exeter software*, NY, USA). Dendrograms were constructed by the method of UPGMA (*Unweighted Pair-Group Method Arithmetic Average*) in the NTSYS pc-programme.

Results and discussion

Effects of salinity on growth

Increasing NaCl concentrations in the nutrient solution resulted in a reduction in leaf area (Figure 1) and dry weight (Figure 2) for most genotypes. The effect, common in glycophytes (salt sensitive plants), had been previously recorded for different banana genotypes (Borges and Cintra 1988, Araújo Filho *et al*. 1995). The

reduction in leaf growth was due partly to a reduction in the net assimilation rate of CO_2 (Akita and Cabuslay 1990) as a result of the closure of the stomata which was in response to the low potential of water in the soil or substrate, itself the result of an increase in salt concentration. There is a metabolic cost to adaptation to salinity as a result of the diversion of part of the metabolic energy to the compartmentalization of ions and synthesis of organic solutes (Binzel *et al*. 1985). With treatment at 100 mol/m^3 NaCl, the biggest reduction in leaf area occurred with 'FHIA-18' (51.34% reduction) followed by 'Calcutta 4' (49.64%) and 'Nanicão' (45.70%). In contrast, leaf area in 'Caipira' and 'Pacovan' was reduced only by 25.14% and 28.91% respectively (Figure 1). With 'Caipira', the reduction occurred on a leaf whose area was already small in the control (without NaCl) treatments.

Stress symptoms such as chlorosis and necrosis of the margin of the leaf lamina were more severe in the diploid 'Calcutta 4' up to the time of leaf death. The symptoms reduced the photosynthetically active area and markedly reduced growth.

In non-saline conditions, 'Nanicão' and 'Pacovan' had the highest dry weight (50.76 g/plant and 49.80 g/plant respectively) as shown in Figure 2. Furthermore, at the highest salinity (100 mol/m^3 NaCl) 'Pacovan' stood out with the lowest reduction in dry matter production (40.01%), whereas for 'Nanicão' and 'Calcutta 4' the reduction was 58.95% and 69.32% respectively. 'Pacovan' maintained a high production of biomass at the highest level of NaCl (100 mol/m^3), a characteristic that is important for the selection of stress-tolerant genotypes. In 'Pacovan', maintaining 60% of the biomass coincided with a small reduction in leaf area in comparison with other genotypes.

Accumulation of ions

There were no significant differences between genotypes as regards the concentration of sodium in the roots and rhizome (Table 1). However, 'Pacovan' and 'FHIA-18' accumulated less sodium in the pseudostem when subjected to 100 mol/m^3 NaCl in comparison with the other genotypes studied (Table 1). 'Pacovan' and 'FHIA-18' also stood out by having, together with 'Caipira', the lowest concentrations of sodium in the leaf lamina when grown at the highest saline conentration (Table 1). In comparison, 'Calcutta 4' accumulated more sodium in the leaf lamina than the other cultivars, with concentrations up to eight times higher than the untreated control. These results show a genetic difference in the capacity to exclude sodium from the aerial parts, a process which avoids the increase of sodium concentration in the leaves and hence minimizes the toxic effects on leaf metabolism, particularly photosynthesis (Boursier *et al*. 1987). Considering that excess sodium in the medium induced passive accumulation of the ion in the root and that the root has a finite capacity to act as a site for accumulation (sink), there is probably a mechanism to exclude sodium that operates at root level and avoids the translocation of sodium to aerial parts. Similarly, the mechanism for the translocation of sodium to the leaves, and the mechanism for absorption at the level of the root appear to be regulated separately. The lack of ability to control sodium concentrations in metabolically active tissue, such as leaves, induces serious physiological and biochemical disturbance (Boursier and Lauchli 1990, Botella *et al*. 1997).

Of the various ions, chloride accumulated the most in vegetative tissues. 'Calcutta 4' showed the highest concentration of chloride in the leaf lamina when treated with

Table 1. Sodium, chlorine, potassium and calcium content of pseudostem, roots + rhizome and leaf lamina in five genotypes of banana subjected to 21 days treatment with NaCl.

	Pseudostem			Root + rhizome			Leaf lamina		
	NaCl (mol/m^3)			NaCl (mol/m^3)			NaCl (mol/m^3)		
	0	50	100	0	50	100	0	50	100
Genotypes	Na^+ (g/kg)			Na^+ (g/kg)			Na^+ (g/kg)		
Caipira	1.6bA*	10.4abA	15.4aA	2.9aA	8.8aA	12.9aA	3.5aB	5.1bAB	6.5cA
Pacovan	2.1aB	9.2abA	10.8bA	4.6aA	10.0aA	15.4aA	3.2aB	5.3bA	6.3cA
Calcutta 4	1.6aC	7.9bB	13.8aA	2.9aB	9.2aAB	15.4aA	3.8aC	18.1aB	28.0aA
FHIA-18	0.8aB	6.7bA	7.9bA	2.9aA	8.8aA	11.7aA	3.4aB	5.5bA	5.8cA
Nanicão	1.7aB	12.1aA	12.5aA	4.7aB	13.3aAB	16.7aA	3.5aC	5.8bB	14.9bA
	Cl^- (g/kg)			Cl^- (g/kg)			Cl^- (g/kg)		
Caipira	4.0aB	53.4abA	62.1aA	3.5aB	41.4aA	49.7aA	3.3aB	40.6aA	42.2bcA
Pacovan	4.8aB	37.6bA	42.6aA	5.7aB	41.8aA	45.9aA	3.9aB	31.4aA	34.3cA
Calcutta 4	3.1aB	44.3abA	49.3aA	5.3aB	45.1aA	50.5aA	4.9aC	35.2aB	66.2aA
FHIA-18	3.5aB	48.0abA	46.8aA	4.0aB	38.5aA	43.4aA	5.6aB	29.9aAB	38.1cA
Nanicão	6.1aB	62.9aA	57.9aA	3.1aB	46.7aA	45.1aA	4.3aB	37.7aA	58.8abA
	K^+(g/kg)			K^+ (g/kg)			K^+ (g/kg)		
Caipira	117.1aAB	138.8abA	97.9abB	54.2aA	64.2aA	55.5aA	25.8abA	27.5aA	30.0abA
Pacovan	90.4aB	145.4aA	117.9aAB	57.9aA	52.9aA	61.3aA	20.8bA	26.3aA	25.8bA
Calcutta 4	99.2aA	113.3abA	73.8bB	54.2aA	54.2aA	52.5aA	28.8aA	16.3bB	11.7cB
FHIA-18	95.0aA	104.2bA	106.3abA	41.3aB	62.9aA	60.8aA	25.8abB	30.4aAB	32.5aA
Nanicão	100.8aB	131.3abA	107.5abB	54.6aA	58.3aA	66.7aA	24.2abA	27.1aA	22.9bA
	Ca^{2+} (g/kg)			Ca^{2+} (g/kg)			Ca^{2+} (g/kg)		
Caipira	4.6aA	5.7bA	6.7bA	5.3bA	4.7bA	6.6abA	10.6abA	8.3aB	6.7abB
Pacovan	4.2aA	5.0bA	3.7cA	8.0abA	6.2bA	6.1abA	7.6cA	5.5bcB	4.4bB
Calcutta 4	6.4aB	11.5aA	13.2aA	8.2abA	9.9aA	8.7aA	11.7aA	4.3cB	4.7bB
FHIA-18	6.8aA	6.1bA	5.9bcA	7.5abA	5.5bA	4.8bA	9.3bcA	6.2bB	4.9bB
Nanicão	6.6aA	5.9bA	7.2bA	8.8aA	5.8bB	7.3abAB	9.7bA	7.4abB	8.4aAB

* Values in the same column followed by the same lower case letter, or by the same upper case letter in the same row, are not significantly different according to Tukey's test at a 5% level of probability.

100 mol/m³ of NaCL. With the same treatment, 'Pacovan' and 'FHIA-18' maintained the lowest levels of chloride in the leaf lamina (Table 1). Ionic compartmentalization at the level of the organ is a mechanism for tolerating sodium stress, however the physiological basis is still unclear (Shanon and Noble 1995). Ions accumulate in the leaves as a result of the quantity of lost water through transpiration. In leaves, however, concentrations of ions that exceed the tolerance of the plant induce characteristic symptoms such as necrosis and scorch. Symptoms of saline stress in the leaf lamina were more severe in 'Calcutta 4' than in the other genotypes studied and, furthermore, coincided with the highest concentrations of Na^+ and Cl^- in this cultivar.

'Calcutta 4' showed a significant reduction in K^+ in the aerial parts (leaf lamina and pseudostem) with increased salinity (Table 1). The reduction was accompanied by an increase in Na^+ in the tissue. Tolerance to saline stress was associated with the exclusion of toxic ions and the selectivity of the membrane, mainly discrimination between Na^+ and K^+ (Botella *et al.* 1997), a mechanism that was absent in 'Calcutta 4'. As regards the distribution of K^+ in different parts of the plant, the highest K^+ content was always found in the pseudostem in all the genotypes studied (Table 1).

The increase in salinity coincided with a reduction in Ca^{2+} in the leaf lamina in all genotypes studied, except for the diploid 'Calcutta 4', which showed an accumulation of Ca^{2+} in the pseudostem, with both salt treatments (Table 1).

The different results between the genotypes studied were related to the capacity for adaptation and growth in a saline medium, which is controlled by genetic factors. Nevertheless, the cultivar 'Nanicão', showed an initial vigour similar to the cultivar 'Pacovan', but the latter showed more productivity and tolerance under conditions of imposed saline stress. High biomass production in 'Pacovan' was attributed to a capacity to restrict the movement of Na^+ and Cl^- ions to the leaves, ensuring a reduced symptom expression and reduced damage to the leaf lamina. In comparison, when subjected to saline stress, the diploid 'Calcutta 4' showed higher Na^+ and Cl^- contents in the leaf lamina that were associated with the severest symptoms of toxicity to these ions and the lowest biomass production.

Isoenzymes

The esterase isoenzyme profile did not produce consistent bands for any treatment of the genotypes studied. In contrast, Reyes *et al.* (1998) detected a total of 14 bands, distributed in 5 zones of enzymatic activity, when they analyzed leaves of 15 clones, originating from *in vitro* cultivation, of the genus *Musa*. This suggests that the bands could be activated at other stages of plant development.

The highest amount of polymorphism occurred with the peroxidase profile (PO), with a total of 15 bands (Figure 3). Of these, three (Po-10, Po-11 and Po-15) were revealed in all the individuals analyzed, independently of treatment. Only in the treatment with salt were the following bands seen: Po-4 and Po-7 in 'Pacovan', Po-6 and Po-7 in 'Nanicão' and Po-14 in 'Calcutta 4'. The presence of salt probably inactivated the action of these enzymes. In contrast, a concentration of 100 mol/m³ of NaCl activated the Po-2, Po-8, Po-13 and Po-14 bands in 'FHIA 18', and the Po-5 band in 'Pacovan'. In spite of the increased degree of polymorphism shown in the system, bands were not detected simultaneously in both saline treatments. Other researchers (Jarret and Litz 1986, Bhat *et al.* 1992a) working with other genotypes recorded various very polymorphic zones of activity in banana.

Four bands appeared in the shikimic dehydrogenase profile (SKDH). The Skdh-3 and Skdh-4 bands showed in all the individuals analyzed (Figure 3). The activity of this system has also been described in other work with different banana genotypes (Jarret and Litz 1986, Horry 1989, Espino and Pimentel, 1990, Bhat *et al.* 1992b, Reyes *et al.* 1998). The Skdh-1 band showed only in the genotype 'Calcutta 4' when it was subjected to salt. In the 'FHIA-18' and 'Calcutta 4' genotypes, the presence of salt activated the Skdh-2 and Skdh-1 bands, respectively, which suggests that activation of these enzymes, in these genotypes, may be related to saline stress.

The malate dehydrogenase profile (MDH) showed three bands of activity (Mdh-1, Mdh-2 and Mdh-3) in all individuals, demonstrating that the system was monomorphic (Figure 4).

Two regions of bands were observed in the glutamatic oxaloacetic transaminase profile (GOT). Band Got-2 occurred in all individuals (Figure 4). Band Got-1 showed no activity in 'FHIA-18' with the no-salt treatment nor in 'FHIA-18' and 'Calcutta 4' treated with 100 mol/m³ NaCl. The low degree of polymorphism shown in this system was insufficient to interpret the relationship to saline stress.

The acid phosphatase profile (ACP) showed five bands, the most anodic, Acp-5, showing in all the individuals analyzed (Figure 4). Bands Acp-4 and Acp-3 were present only in 'Pacovan' when not subjected to saline stress. With this treat-

Figure 3. *Zymogram of isoenzyme variation of peroxidase (PO) and shikimic dehydrogenase (SKDH) for the genotypes studied (migration to anode). Column 1: treatment without salt, column 2: treatment with 50 mol/m³ NaCl, and column 3 treatment with 100 mol/m³ NaCl.*

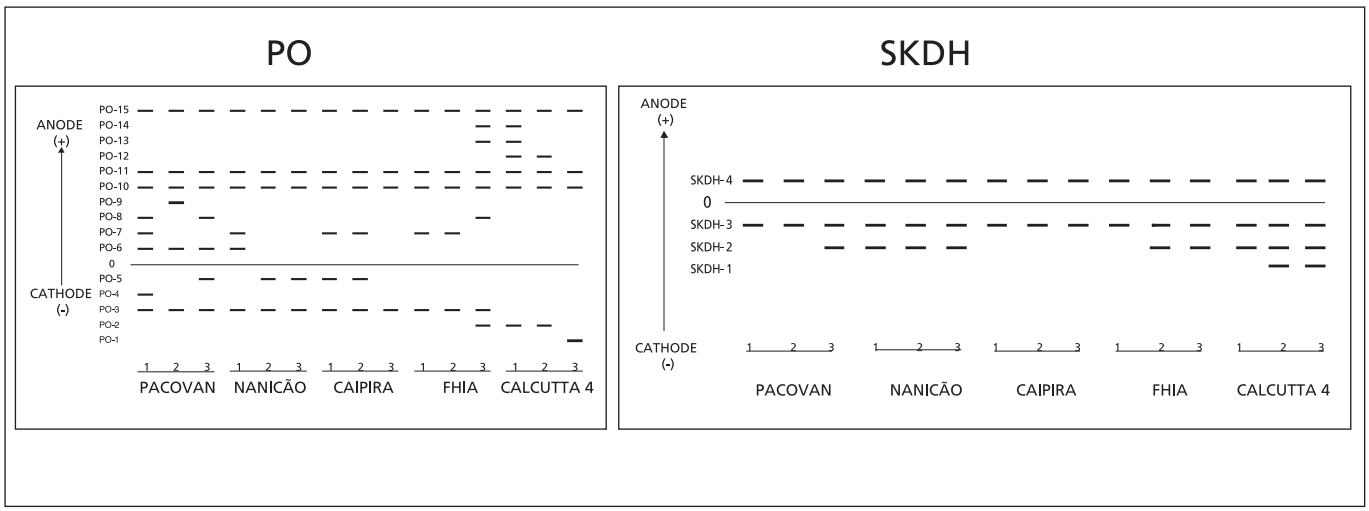
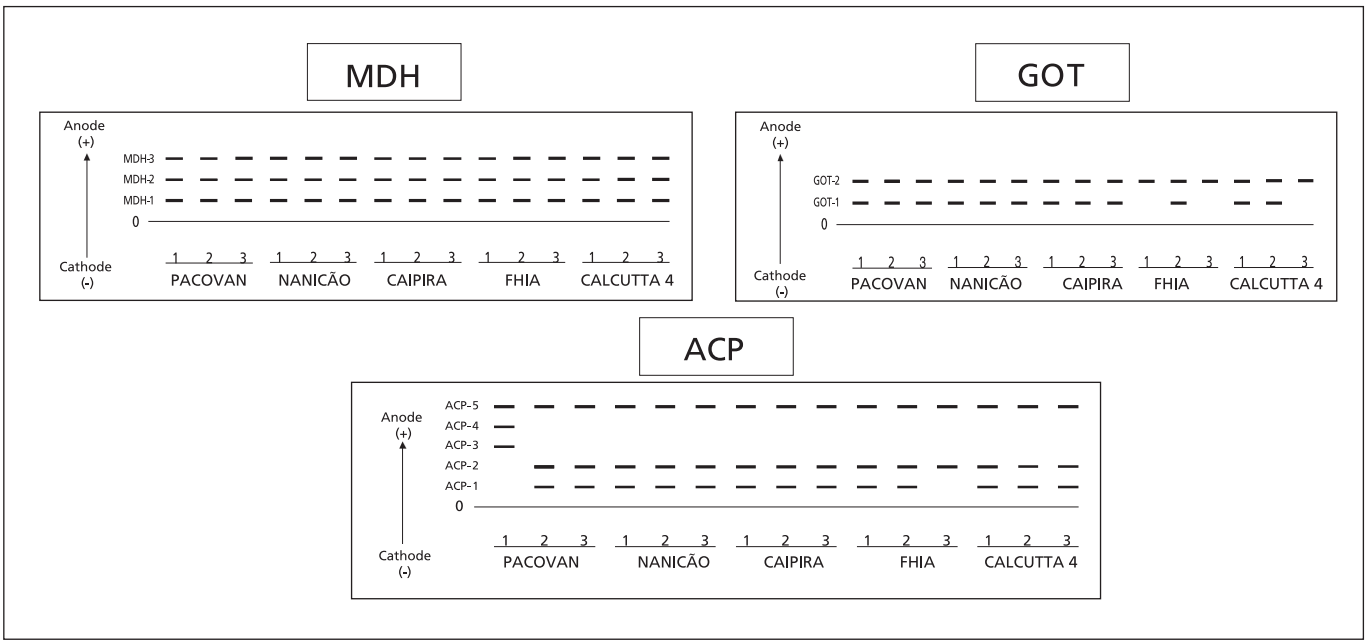


Figure 4. *Zymogram of isoenzyme variation of malate dehydrogenase (MDH), glutamatic oxaloacetic transaminase (GOT) and acid phosphatase (ACP) for the genotypes studied (migration to anode). Column 1: treatment without salt, column 2: treatment with 50 mol/m³ NaCl and column 3: treatment with 100 M NaCl.*



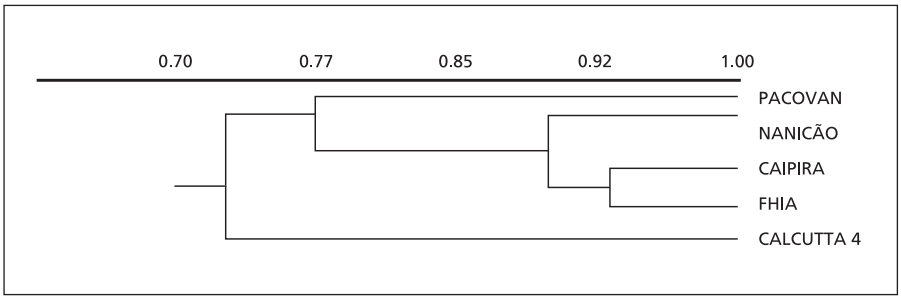
ment, 'Pacovan' did not show band Acp-2, which appeared in all other individuals, or Acp-1, which also was not detected in 'FHIA-18' with treatment at 100 mol/m³ NaCl. This isoenzyme system can be used as a molecular maker for 'Pacovan', since, in the presence of salt (50 and 100 mol/m³), two bands did not show activity (Acp-3 and Acp-4) and the salt activated the expression of bands Acp-1 and Acp-2. With genotypes that possess only genome group A ('Nanicão', 'Caipira' and 'Calcutta 4'), the same pattern of bands was observed independently of the presence or absence of salt.

Genotypes with distinct levels of ploidy (diploid, triploid and tetraploid) were evaluated, therefore the failure to identify a marker for sensitivity to salt probably resulted from the wide range of genetic variability, as well as from the numbers of genes involved in the activation of enzymes related to metabolic pathways activated by saline stress.

Of the five isoenzyme systems that showed activity, four showed bands that were polymorphic (ACP, GOT, PO and SKDH). A total of 244 bands were observed, 150 of which were monomorphic and 94 polymorphic. According to the analyses of genetic diversity, the most closely related genotypes were 'Nanicão' (AA) and 'FHIA-18' (AAAB) with a similarity grade of 0.969 (96.9%); the most distant genotypes were 'Pacovan' (AAB) and 'Calcutta 4' (AA), with a similarity grade of 0.606 (60.6%).

Physiological and biochemical analysis showed that 'Calcutta 4' was the most sensitive to salt and 'Pacovan' the most tolerant. The dendrogram

Figure 5. *Analysis of grouping of individuals from genotypes 'Pacovan' (1, 2, 3), 'Nanicão' (4, 5, 6), 'Caipira' (7, 8, 9), 'FHIA-18' (10, 11, 12) and 'Calcutta 4' (13,14, 15), obtained with the programme NTSYS-pc (option UPGMA).*



(Figure 5) clearly showed that genotypes can be divided into two groups: one group formed by the genotype 'Calcutta 4', which is diploid and sensitive to salt and, the other group comprising the remainder of the genotypes, which were triploids (AAA and AAB) and tetraploids (AAAB). ■

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Breeding | **Somaclonal variant**

'J.D. Dwarf': a superior Cavendish cultivar?

J. Daniells

Bananas and plantain represent the number one fruit crop in the world, both in terms of production, around 98 millions tonnes, and trade, valued at over US$4306 million (FAOSTAT database 2002). Cultivars belonging to the Cavendish subgroup (AAA) account for 41% of world production, of which one-third (or 13% of world production) is exported (INIBAP 1999).

The Cavendish cultivars have dominated the export trade since the demise of 'Gros Michel' over 50 years ago thanks to their high yields, long transport life and widely accepted flavour. Their main drawback is their susceptibility to a wide range of pests and diseases, in particular black leaf streak disease(*Mycosphaerella fijiensis*). As a result, they require regular applications of pesticides. Efforts by research organizations have been underway for several years to find a replacement that would have equivalent features as the Cavendish cultivars but would be less dependent on pesticides.

Several Cavendish cultivars, like 'Grande naine', 'Williams', 'Valery', 'Poyo', 'Robusta' and 'Giant Cavendish', are grown all over the world, each having its own advantages and disadvantages. For example, the 'Grande naine' cultivar tends to be less prone to wind damage but does not fare as well as taller cultivars, such as 'Robusta' and 'Valery', in poorer soils and drier conditions. The main cultivar grown in Australia is 'Williams', which occupies over 10 000 hectares.

In 1988, the Queensland Department of Primary Industries (QDPI) came up with a variety, 'J.D. Dwarf', which may be of interest to smallholders around the world. 'J.D. Dwarf' was collected as an offtype in 1988 in a tissue culture planting of the 'Williams' cultivar. Data were collected on a few plants grown at the South Johnstone Research Station and trials were established on growers' properties in 1990. The cultivar was released to the industry in 1998 for more widespread on-farm evaluation (Daniells and Bryde 1998). It is now grown on about 100 ha in northern Queensland.

Research station observations

The original selection was very dwarf in habit and had unusually upright leaves but in subsequent plantings, the dwarf habit was much less pronounced. Compared to 'Williams', 'J.D. Dwarf' is not dramatically different in height. As shown by our limited trial results, 'J.D. Dwarf' tends to be marginally taller in mother plant crops and slightly shorter in ratoon crops (Table 1).

The fruit of 'J.D. Dwarf' tends to be about 5% shorter than 'Williams'. This could impede the adoption of 'J.D. Dwarf' by wholesalers and retailers who tend to pay premium prices for longer fruits, even if consumer surveys indicate a preference for fruits of intermediate length. This difference in length would be of lesser consequence for smallholders producing for home consumption and domestic sales.

When harvested at the same finger diameter, bunches of 'J.D. Dwarf' weighed less than 'Williams', although the difference was not significant. However, 'J.D. Dwarf' bunches can be allowed to fill out more so an extra week of fruit filling can make up for the otherwise reduced bunch weight.

On-farm observations

'J.D. Dwarf' has a sturdier pseudostem than 'Williams' (Figure 1), a feature which makes it more resistant to wind damage, the single most important cause of yield loss in banana plantations (Stover and Simmonds 1987). The greater wind resistance of 'J.D. Dwarf' should reduce some of these losses. This should translate into economic benefits for commercial growers and enhanced food security for subsistence farmers. 'J.D. Dwarf' can also often be grown without any form of bunch support such as twine or wooden/bamboo props. Another contributing factor to its stability is its relatively upright pseudostem which seldom leans. However, special care needs to be taken during harvest. When the pseudostem of 'J.D. Dwarf' is cut with a cane knife or machete, the pseudostem can collapse as the bunch is lowered onto the shoulders of the person harvesting.

Maturity staining is a physiological disorder of the fruit which is characterized by bronze-red blemishes on the peel. It causes major production losses in northern Queensland (Daniells 1985) and in some places like Costa Rica (Lahav *et al.* 2000). Growers' observations indicate that 'J.D. Dwarf' is less prone to maturity staining. The fruit of 'J.D. Dwarf' can be allowed to fill out more with much less maturity staining compared to 'Williams'.

During the cooler winter months of northern Queensland, fruit chilling in the field is a significant problem (Daniells 1997). The same is true of other marginal production areas located away from the equator. Fruits of 'J.D. Dwarf' tend to be less sensitive to chilling than 'Williams'. Consequently, it has a good bloom and a nice yellow colour during ripening, provided temperatures do not plunge too low. The growers in our trials consistently received better prices for 'J.D. Dwarf' because of its better 'bloom'.

The upright leaves of 'J.D. Dwarf' (Figure 1) may explain the better fruit bloom and lesser maturity staining because of the resulting better light regime in the canopy. 'J.D. Dwarf' may have a higher optimum plant density, but this requires investigation.

Table 1. Yield and plant characteristics of J.D. Dwarf at South Johnstone Research Station and cooperating growers' properties.

	Days from planting to bunch harvest	Bunch weight (kg)	Finger length of second hand (cm)	Pseudostem height (cm)	Leaf lamina length (cm)	Leaf lamina width (cm)	Length to width ratio of leaf lamina
South Johnstone Research Station[a]							
J.D. Dwarf	351 ± 4 [b]	22.9 ± 1.4	24.5 ± 0.5	214 ± 4	195 ± 6	89 ± 1.9	2.20 ± 0.03
Williams	363 ± 5	24.9 ± 0.7	25.9 ± 0.5	205 ±4	195 ± 3	84 ±1.3	2.34 ± 0.02
Ratoon #2 [c]							
J.D. Dwarf	908 ±10	40.0 ± 2.1	25.6 ± 0.5	303 ±7	252 ± 7	101 ±1.7	2.49 ± 0.04
Williams	952 ± 21	44.0 ± 2.0	27.1 ± 0.5	327 ± 20	265 ± 13	98 ± 2.9	2.69 ± 0.08
Growers' properties [d]							
J.D. Dwarf	400	26.1	24.0	237	n.a.	n.a.	n.a.
Williams	403	27.3	24.8	232	n.a.	n.a.	n.a.

[a] Data are the means of six plants.
[b] Standard error of the mean.
[c] Data for first ratoon have been excluded because of wrong sucker selection.
[d] Data are the means of four locations with 20 plants per variety at each location; only data on plant crop are available from growers.

Some growers have found that 'J.D. Dwarf' is easier to pack in the existing 13 kg fibreboard cartons. This is because the fruit of 'J.D. Dwarf' fills out right to the tip - there is no pinching on the flower end. Thus there is more weight for a given length of fruit, and even more so if bunches are allowed to fill out. J.D. Dwarf also has well shaped hands, which further facilitates packing.

In northern Queensland, emerging bunches, when still upright, are routinely injected with insecticide for the control of the banana scab moth *(Nacoleia octasema)*, and incidentally for the control of flower thrips (*Thrips hawaiiensis*). But because the leaves of 'J.D. Dwarf' are so upright, it is more difficult to detect and easily inject the emerging bunch. Ground application of fungicides can also be hampered by its upright leaf habit.

Bacterial corm rot caused by *Erwinia* spp. is a growing problem in northern Queensland (Daniells 1995). Losses from plant fallouts have been as great as 20-30% in some first ratoon crops. Indications so far from growers are that 'J.D. Dwarf' is more susceptible than 'Williams' to corm and heart rots. 'J.D. Dwarf' may be more sensitive to environmental stresses making it more vulnerable to invasion by this opportunistic pathogen (Buddenhagen 1994). Ongoing studies may identify antagonistic microorganisms to inoculate tissue culture plantlets.

So far, field plantings of 'J.D. Dwarf' established from tissue culture have been reasonably true-to-type, with less than 3% offtypes on average. Interestingly, most of these offtypes are a throw back to 'Williams' from which the cultivar is derived. If other clones produced as few offtypes as 'J.D. Dwarf', growers would be more appreciative of tissue culture.

Conclusion

The origins of 'J.D. Dwarf' highlight the value of collecting and conserving germplasm variants of tissue culture plantings. It also highlights the need for the scientific community and growers to be on the lookout for any variation which might have potential benefits (Daniells and Smith 1993). Now, more than ever, banana growers need to exploit variations for the benefits they may bring.

As to whether 'J.D. Dwarf' is a superior Cavendish cultivar, the answer will depend on local circumstances. Daniells (2000) has argued that there is no perfect variety, that each has its own set of advantages and disadvantages. Growers must find out what is best for them. Those wishing to evaluate 'J.D. Dwarf' should contact INIBAP. ■

***Figure 1.** 'J.D. Dwarf' (right) has more upright and slightly wider leaves than 'Williams' (left).*

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Evaluation of the progeny from a cross between 'Pisang Berlin' and *M. acuminata* spp. *burmannicoides* 'Calcutta 4' for evidence of segregation with respect to resistance to black leaf streak disease and nematodes

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The breeding process to obtain resistant cultivars can be accelerated if the presence of the desired plant characteristic, such as resistance to a fungal disease or nematodes, can be demonstrated at an early plant stage. This is possible when a link can be found between the presence of certain DNA sequences, the so-called molecular markers, and the desired plant characteristics. These DNA bands can be identified in segregating populations, i.e. when segregation has occurred among the progeny derived from a cross between two unlike parents, in this case a resistant and a non-resistant one.

This study is part of a project to find molecular markers linked to resistance to black leaf streak disease (commonly known as black Sigatoka) and nematodes. 'Pisang Berlin', the male parent, was chosen because of its susceptibility to black leaf streak disease while 'Calcutta 4' was chosen because its hypersensitive reaction to black leaf streak disease made it resistant to the pathogen.

Materials and methods

Pollen from 'Pisang Berlin' was crossed on female flowers of *Musa acuminata* spp. *burmannicoides* 'Calcutta 4'. The resulting 155 seeds were put in water during 12 hours and the floating seeds, indicating non-functionality, were eliminated. The 150 viable seeds were disinfected during 10 minutes in a 3% calcium hypochlorite solution and washed with sterile water. The embryo of each seed was extracted under aseptic conditions and placed in Murashige and Skoog medium. In the following 45 days, 120 of the 150 extracted embryos germinated in a growth chamber (27 ± 1°C, 12 hours light, relative humidity of 80%). From the germinated plantlets, 10 were randomly selected and micropropagated. Seven clones of each plantlet (line) were grown in plastic bags in the greenhouse to serve as planting material.

Field experiment

The experiment was established at the CORBANA research station in 28 Millas, canton Matina, in the Atlantic coastal plain of the province of Limón, Costa Rica. Accumulated rainfall between June 2000 and September 2001 reached 5089 mm. June 2001 was the wettest month (548 mm of rain) and March 2001 the driest (76 mm of rain). Average maximum/minimum temperatures over this period were 29.5°C/20.2°C. The soil was a sandy clay (52% sand, 42% clay and 6% lime), with a pH of 5.9, an organic matter content of 1.3%, and a bases content 25.7 cmol/L of Ca, 10.1 cmol/L of Mg, 0.24 cmol/L of K, 7.2 mg/L of P, 269 mg/L of Fe, 5 mg/L of Cu, 1.1 mg/L of Zn and 54 mg/L of Mn.

Before starting the experiment, a site 20 x 40 m was planted with *Musa* AAA cv. 'Grande naine' to assure a high presence of plant parasitic nematodes, especially *Radopholus similis*. Distance was 2.5 m between rows, and 2.7 m between plants. East-west oriented small ridges about 0.5 m wide and 0.2 m high were made. They ended in a central channel to evacuate surface water. The 70 plants (10 lines x 7 repetitions/line) were planted in groups of 1 or 2 plants per line at 4 different dates over a period of 5 weeks, to ensure the availability of male and female flowers for the pollination of F2 within each line.

Fertilizer (Nutrex 3%) was applied weekly to the leaves for three weeks and to the soil (DAP and a mixture of N-P_2O_5-K_2O-S at 16.3-3.6-28.9-3.7), ranging between 30 and 180 g per month. Weeds were cut manually every four weeks. Plants were desuckered at flowering, to observe the number of suckers produced. No fungicides or nematocides were applied.

Before planting, and every six weeks after planting, mixed roots from the mother plant and the suckers of three randomly selected plants of all lines were sampled to determine the numbers of nematodes present. Sampling per line was not possible because of the small amount of plants. At the time of flowering and at harvest, morphological parameters like plant height, third leaf width, circumference at 1 m, bunch weight and following sucker height were measured. Also a series of banana descriptors (IPGRI 1996) were noted at flowering and at harvest for the plants of each F1 line.

Disease severity, or the amount of leaf area affected by black leaf streak disease was evaluated in all plants. Disease severity was expressed as a grade using Gauhl's modification of Stover's scale (Gauhl 1989). The following parameters were also calculated: total functional leaves, youngest streaked leaf, youngest spotted leaf and infection index. This index was obtained by adding the results of multiplying the number of leaves in each grade of the modified Stover scale with their respective grade of infection, and dividing this sum by the number of evaluated leaves in the plant in question.

Pot experiment

At least 30 plantlets of each of the 10 selected F1 lines from the previously mentioned cross were multiplied *in vitro*. After hardening, plantlets were put in 1.8 L pots with sterilized local banana soil as substrate. The soil was a sandy clay (54% sand, 39% clay and 7% lime), with a pH of 6.2, an organic matter content of 5.6%, and a bases content of 7.2 cmol/L of Ca, 1.7 cmol/L of Mg, 0.8 cmol/L of K, 4 mg/L of P, 54 mg/L of Fe, 5 mg/L of Cu, 1.4 mg/L of Zn and 30 mg/L of Mn. Plantlets received a complete nutrition solution (Hoagland and Arnon 1950) every 2-3 days, and the same solution was sprayed daily on the leaves. After 3 weeks of adaptation in pots, plantlets of each line were divided in 2 groups of 12 replications, one group for future inoculation and the other for use as non-inoculated control.

R. similis was reared in the dark on surface-sterilized carrot disks in petri dishes in a growth chamber at 28°C (Speijer and De Waele 1997). Once the numbers observed on the inner surface of

the dishes was high, the nematodes were washed into a beaker, water was added up to a volume of 200 ml, and the number present in a volume of 2 ml was counted under a microscope. Based on the number of females, the volume needed to obtain 500 female *R. similis* was calculated. The suspension was applied in 5 holes, 0.5 cm in diameter by 1.5 cm in depth, made at about 1 cm from the base of the pseudostem. A small quantity of water was applied afterwards. Eight weeks later, plants were removed from the pots, roots were washed gently and fresh shoot and root weight was measured. Roots were cut in 2 to 3 cm pieces and liquefied in a kitchen blender at low and high speed for 10 sec each. The root suspension was washed over a set of nested sieves (0.25, 0.106 and 0.025 mm), and the material recovered from the last sieve was put into a 200 ml beaker. The suspension was homogenized with an air bubble injector and the number of nematodes counted was expressed per 100 g of roots.

In both field and pot trials, the experimental layout was a complete randomized design. The variables measured in the different F1 lines were compared, using analysis of variance, with PC-SAS (SAS Version 6.12 for Windows, SAS Institute Inc., Cary, USA). Before statistical analysis, nematode numbers were $\log_{10}$ (x+1) transformed, and means were evaluated by Waller-Duncan's K-ratio T test.

Results and discussion

Field experiment

When measuring plant characteristics, only the width of the third leaf ($P< 0.001$), the circumference at 1 m at flowering ($P= 0.0001$) and bunch weight in the first generation ($P= 0.0002$) differed between tested lines. Only little variation was observed for flower and bunch parameters.

In the evaluation of the susceptibility of these lines to black leaf streak disease, differences in the infection index between tested lines were recorded (Table 1). Differences in the index between the progeny lines suggest segregation in the first and second generations. However, in the absence of data on the parents, segregation with respect to resistance/susceptibility cannot be confirmed.

As for resistance to nematodes, although the number of *R. similis* was high before the start of the experiment (7600 per 100 g of roots), no nematodes were found in the root system one year later, suggesting a certain level of resistance of the F1 lines to *R. similis*.

Table 1. Infection index for black leaf streak disease over two production cycles of plants from 10 progeny lines resulting from a cross between 'Pisang Berlin' and 'Calcutta 4'.

Progeny lines	Infection index*			
	At flowering		At harvest	
	F1	F2	F1	F2
A	1.48bc**	0.90abc	2.35cd	2.04b
B	1.72ab	1.12a	3.32bc	2.29b
C	1.79ab	0.88abc	3.82ab	2.76ab
D	1.08d	0.58bc	1.59d	2.05b
E	1.77ab	0.66abc	2.40cd	2.16b
F	2.16a	0.98ab	4.10ab	2.54ab
G	1.33bc	0.59bc	2.15cd	2.28b
H	1.75ab	0.73abc	2.93bc	1.93b
I	1.34bc	0.43bc	1.67d	1.79b
J	1.84ab	1.03c	4.62a	3.51a
P	0.0004	0.0001	0.018	0.045

* Each value is the mean of 7 observations

** Numbers followed by the same letter are not significantly different at $P\leq 0.05$, according to Waller-Duncan's test

Table 2. Comparison of the number of *Radopholus similis*/100 g of roots and of the root weight of inoculated and non-inoculated F1 plants resulting from a cross between 'Pisang Berlin' and 'Calcutta 4'.

F1 lines	*Radopholus similis*/ 100 g of roots	Root weight (g)		
		Inoculated	Non-inoculated	Difference (P)
A	37 737b*	18.33ab	31.92abcd	0.013**
B	58 219ab	27.75bcd	44.50cde	0.004
C	163 086a	26.25abcd	61.42g	0.0001
D	90 724ab	19.08ab	30.83abc	0.0001
E	77 215ab	38.83d	45.75de	NS
F	119 535a	22.50abc	42.42cde	0.0001
G	59 912ab	12.50a	23.50ab	0.019
H	70 813a	38.50d	47.17ef	NS
I	2 159c	25.25abcd	35.83bcde	NS
J	114 385ca	36.42cd	60.08fg	0.007
P	0.0001	0.0002	0.0001	

*Each value is the mean of 12 observations that were $\log_{10}$(x+1) transformed for statistical analysis. Values followed by the same letter do not differ significantly at $P< 0.05$, according to Waller-Duncan's method.

** Probability of statistical difference of root weight between plants with and without *R. similis* inoculation. NS= non significant.

Pot experiment

In the pot experiment, the number of *R. similis* per 100 g of roots differed significantly ($P= 0.0001$) among some of the F1 lines (Table 2). The number of nematodes in plants from line I is significantly lower than lines C, F, H and J especially, indicating segregation, but in the absence of data on the parents, segregation with respect to resistance/susceptibility cannot be confirmed. The male parent of the F1 crosses, 'Pisang Berlin', is not generally known to possess resistance to *R. similis*, although Fogain *et al.* (1996) found this variety less susceptible than 'French sombre' (*Musa* AAB). There are also mixed reports from the female parent, *Musa acuminata* ssp. *burmannicoides* 'Calcutta 4'. According to Binks and Gowen (1997), Fogain *et al.* (1996) and Viaene *et al.* (in press), this diploid cultivar showed resistance to *R. similis*. Similarly, the tetraploid TMHx 660K-1, resulting from the crossing of susceptible 'Enzirabahima' (*Musa* AAA-EA) with 'Calcutta 4', was less susceptible to *R. similis* than 'Valery' (*Musa* AAA) (Dochez *et al.* 2000), using the single root inoculation method. As 'Enzirabahima' is susceptible to *R. similis*, the partial resistance of TMHx 660K-1 most likely originated from 'Calcutta 4'. However, when evaluating the roots of sword suckers of established mats of various *Musa* varieties in Uganda, 'Calcutta 4' and 'Valery' were found to be equally susceptible to *R. similis* by Speijer *et al.* (1999). Also Price and McLaren (1996), using roots of fully-grown plants in a field trial in Cameroon, observed no difference in susceptibility to *R. similis* between 'Calcutta 4' and 'Grande naine'.

The existence of lines where inoculation with nematodes led to a decrease in root weight and of others which showed no difference in root weight between inoculated and non-inoculated plants, suggest that segregation has occurred.

The high number of nematodes in the inoculated plants of lines E and H suggest a certain level of tolerance towards *R. similis*.

To confirm these data, F2 plantlets, obtained by self pollination of three F1 lines, will be compared for susceptibility to *R. similis* with the parents 'Pisang Berlin' and 'Calcutta 4' and the reference varieties 'Grand naine' and 'Yangambi km 5' in a future pot experiment.

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Resistance to finger drop of diploid genotypes

O. Nunes de Jesus, S. de Oliveira e Silva, M. Di Credico and H. Souza Rocha

Finger drop is a physiological disorder due to the softening and weakening of the pedicel, which makes the finger separate or fall off during maturation (Semple and Thompson 1988). Finger drop is believed to be associated with fast maturation, precipitated by high temperatures in the maturation chamber (New and Marriot 1974). Tetraploid hybrids are more susceptible to the fall of fingers than triploid cultivars (New and Marriot 1974, Dadzie and Orchard 1997).

The methods used so far to evaluate finger drop in bananas were not reliable, the usual method consisting in shaking the hand and counting the number of fallen fruits. To overcome this problem, researchers at the Embrapa Cassava and Tropical Fruit Crops developed an instrument to evaluate resistance to finger drop in bananas.

Since breeding programmes use diploid bananas as a starting point and great variability exists with regard to resistance to finger drop in this group, we evaluated this character in 21 diploid varieties and synthetic hybrids (AA), seeking to identify the most resistant ones for subsequent transfer of that character to the commercial varieties and hybrids being developed.

Materials and methods

The bunches from 21 genotypes (Table 1) were harvested when the fruits of the first bunch showed ripening signs. Five fingers from each of the first five hands were removed with the pedicel. The fruits were placed on a table, allowing the latex to be drained, then dipped in a solution of Ethephon (2 mg/L) for five minutes to provide uniform maturation. They were then acclimatized in a refrigeration chamber to 21ºC and 95% relative humidity.

Based on the methodology proposed by Silva *et al.* (1999), the following characteristics were analyzed: number of fingers per hand, pollen and seed production, length and diameter of the fingers and the pedicel. Resistance to finger drop, expressed in kgf, was estimated, using the methodology proposed by Cerqueira *et al.* (2000). The mechanized detacher, developed at Embrapa Cassava and Tropical Fruit Crops, is composed of a

penetrometer mounted on a wood chassis in which the pedicel is inserted and held by a cleat coupled to a traction train (Figure 1). As the banana is pulled, the cleat moves the train and pressures a piston. Pressure on the penetrometer ceases when the pedicel breaks. The force at the moment of rupture is noted from the penetrometer gauge.

The experimental design was randomized, 19 replications per treatment for measuring the resistance to finger drop, 25 replications per treatment for the finger characteristics and five replications per treatment to evaluate pollen and seed production, and the number of fingers. Scott and Knott's test was used to compare the means.

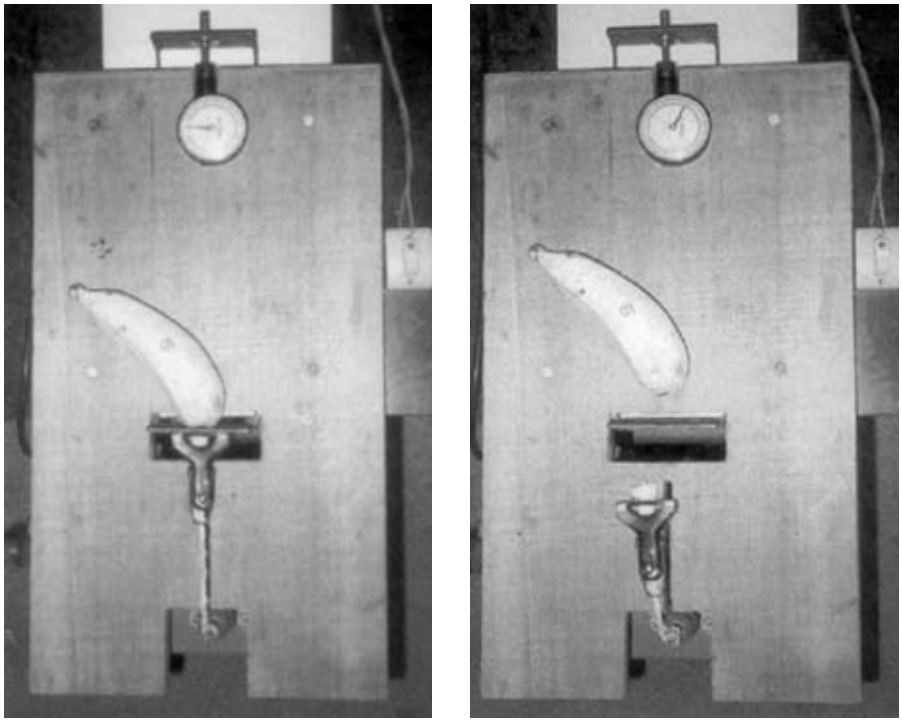

Figure 1. *Apparatus used to measure banana resistance to finger drop.*

Results and discussion

In general, the hybrids had more fingers than the other genotypes (Table 1), given that the selection process to obtain new hybrids eliminated those with low number of fingers (Silva *et al.* 1997). All the genotypes produced pollen (albeit in small amounts), whereas the hybrids tended to produce more seeds than the varieties, making it possible to use these genotypes in breeding programmes. Only one hybrid and four varieties did not produce seeds.

Resistance to finger drop varied from 0.26 to 4.65 kgf (Table 2). The TH03-01 hybrid had the greatest mean resistance to the finger drop and the second longest fingers (Table 2). However, the mean diameter of its fingers, and the mean length and diameter of its pedicels were below the overall average.

The second best diploid in terms of resistance to finger drop was the 'Khai nai on' accession, ex-aequo with the hybrid 4223-06. The mean diameter of the fingers of 'Khai nai on' and the mean length and diameter of its pedicels were above average but the mean length of its fingers was below average, whereas the mean length of the fingers of 4223-06, and the mean diameter of its fingers and pedicels were above average but the length of its pedicels was below average.

The only genotypes which had above average values for all the measured characteristics were the hybrids 0337-02 and 1318-01, and the varieties 'Raja uter' and 'Pipit'. In general, resistance to finger drop was not correlated with finger characteristics. ■

Table 1. Characteristics of the 21 diploid genotypes used in the evaluation of resistance to finger drop.

Genotype	Parents/Country of origin/Donating country	Number of fingers	Pollen production*	Seed production**
TH03-01	Terrinha x Calcutta (*M. acuminata burmannica)*	117	3	2
1741-01	Jary buaya x (Calcutta x Madang)	166	2	2
1318-01	Malaccensis x Sinwobogi	132	4	4
4154-08	(Calcutta x Madang) x (Borneo x Madang)	140	2	2
0323-03	Calcutta x Cultivar *sine nome*	112	3	2
1304-06	Malaccensis x Madang (*M. acuminata banksii*)	164	2	4
4223-06	M53 x Cultivar *sine nome*	108	2	2
0116-01	Borneo (*M. acuminata microcarpa*) x Guyod	137	2	4
0304-02	Calcutta x Madang	103	2	2
0337-02	Calcutta x Galeo	112	3	2
NBA-14	New Guinea	82	2	1
Tongat	Honduras	143	2	1
Khai nai on	Thailand	139	2	1
Pipit	Indonesia	144	5	4
Selangor	France (Guadeloupe)	112	3	2
Khi maeo	Thailand	152	2	2
Tambi	New Guinea	72	3	2
Sowmuk	New Guinea	86	2	2
Niyarma yik	New Guinea	102	2	1
SA	Thailand	126	3	2
Raja uter	Indonesia	100	2	1

* 1=None, 2=Very low (<10%), 3=low (15-30%), 4=average (40-60%), 5=high (>60%)

** 1=None, 2=low (0-10), 3=mean (10-50), 4=high (>50)

Table 2. Mean values of resistance to finger drop and four finger characteristics for the 21 genotypes tested.

Genotype	Resistance to finger drop (kgf)	Finger length (cm)	Finger diameter (mm)	Pedicel length (mm)	Pedicel diameter (mm)
TH03-01	4.65 a	15.28 b	23.24 f	13.48 e	9.16 f
Khai Nai On	3.07 b	9.20 g	28.00 c	15.48 d	10.68 d
4223-06	3.04 b	12.00 d	26.20 d	14.36 e	9.68 e
0337-02	2.82 c	12.32 d	23.80 f	12.36 f	10.52 d
Raja uter	2.71 c	11.90 d	29.92 b	18.16 c	12.18 b
1318-01	2.06 d	12.16 d	25.04 e	15.24 d	9.84 e
Pipit	1.99 d	11.72 e	31.04 a	17.72 c	13.16 a
1741-01	1.78 e	15.88 a	23.64 f	12.28 f	11.44 c
0323-03	1.72 e	13.40 c	26.56 d	10.06 g	12.22 b
1304-06	1.69 e	12.40 d	23.08 f	19.00 b	8.40 f
NBA-14	1.65 e	10.72 f	31.44 a	22.68 a	8.84 f
0304-02	1.57 e	10.32 f	18.08 g	9.88 g	8.84 f
4154-08	1.46 e	12.80 c	22.24 f	10.20 g	9.00 f
Tongat	0.91 f	11.28 e	24.72 e	12.36 f	10.24 d
Khi maeo	0.88 f	8.70 g	23.40 f	19.79 b	5.58 i
0116-01	0.73 f	13.04 c	26.50 d	16.96 c	10.58 d
Nyarma yik	0.72 f	12.06 d	32.28 a	17.66 c	6.36 h
Selangor	0.68 f	7.94 h	19.00 g	15.60 d	6.98 g
SA	0.67 f	13.44 c	31.52 a	17.54 c	11.50 c
Sowmuk	0.62 f	11.36 e	31.60 a	15.66 d	6.24 h
Tambi	0.26 g	9.32 g	18.92 g	13.22 e	6.80 g
Overall average	1.73	11.77	25.73	15.22	9.44

Numbers followed by the same letter are not significantly different at p=0.05 (Scoot & Knott Test).

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Taxonomy | ***Musa* diversity**

Classification and characterization of *Musella splendida* sp. nov.

R.V. Valmayor and Le Dinh Danh

Musella is the most fascinating and unique member of the family *Musaceae*. The type species is *Musa lasiocarpa* as published by A.R. Franchet in Morot, Jour. de Bot 3:329 1889 (Li Hsi-wen 1978). Later, Cheesman (1947), in his monumental series Classification of the Bananas, reclassified it as *Ensete lasiocarpum*, perhaps due to its slightly swollen pseudostem base and persistent flower bracts. But Simmonds (1962) reverted its classification back to *Musa*, arguing that being rhizomatic and polycarpic, *Musella* does not rightfully belong to the genus *Ensete*. Finally, Wu (1976), working with an indigenous specimen in its natural environment in China, realized that the plant cannot indeed be classified either under *Musa* or *Ensete* and adapted the name *Musella lasiocarpa*, a new genus under the family *Musaceae* (Li Hsi-wen 1978).

Geographic distribution

Musella lasiocarpa was described by Franchet (Li Hsiwen 1978) as early as 1889. Ornamental nurseries in Western Europe and the USA have extolled its beauty, its hardiness and resistance to cold temperatures, and its adaptability in the home garden due to its small size. But until today, *Musella* are mostly found only in arboreta and botanical gardens. So far, only one species has been named to the genus. It can be considered, therefore, as the world's rarest banana.

Musella lasiocarpa is indigenous to Yunnan province in South China, primarily at elevations from 1500 m to 2500 m. Botanists in Yunnan are concerned that it may be extinct in the wild because it is now found only as an ornamental in courtyards and under cultiva-

tion by farmers in Yunnan and neighbouring Guizhou and Szichuan provinces in China. The pseudostem is fed to animals as fodder, while the flowers have medicinal value. Recently, *Musella* was discovered growing in the forest of Ha Giang province in neighboring northern Vietnam (Danh *et al*. 1998).

Plant habit of *Musella lasiocarpa*

"Small plant, less than 60 cm tall. Leaf sheaths persistent, pseudostem base only about 15 cm in diameter. Leaf blade elliptical, up to 50 cm long and 20 cm wide with pointed tip and waxy. Inflorescence is erect, densely arranged, 20 to 25 cm long. Bracts are yellow, each subtending 4 to 5 flowers. Female flowers are borne at the base of the inflorescence and male flowers at the top. Fruits are round-ovate with 3 ridges, hairy, 3 cm long and 2.5 cm wide. Inside are 6 seeds, round in shape and dark brown in color" (Figure 1). English translation of original Chinese description from Wu (1976).

Banana collection and conservation in Vietnam

The genus *Musa* is indigenous to Southeast Asia. Several banana prospection and conservation missions have explored and collected *Musa* germplasm in Indonesia, Malaysia, Thailand and the Philippines. Banana explorers in Papua New Guinea and India, particularly in the northeastern region of Assam and its neighboring states, have discovered very interesting germplasm, resulting in revisions of the present theory on the origin and evolution of bananas.

The *Musa* genetic resources of Vietnam have never been thoroughly explored and classified due to the country's extended struggle for freedom. In 1993, the International Network for the Improvement of Banana and Plantain (INIBAP), through its Asia and Pacific Network, then based at the Philippine Council for Agriculture, Forestry and Natural Resources Research and Development (PCARRD), Los Baños, Laguna, Philippines, approved a grant to the Plant Genetic Resources System of Vietnam headed by Dr Nguyen Danh Khoi. In 1994, the first of five prospection missions started to systematically collect, evaluate and conserve *Musa* germplasm throughout the country. Director Le Dinh Danh of Phu Ho Fruit Research Center was appointed leader of the banana collection team. The project yielded 107 accessions, 88 cultivars and 19 wild species. Among the wild and

Figure 1. *Illustration of* Musella lasiocarpa *showing a small plant. Leaves are elliptical (length-width ratio<3), flower bud deltoid with markedly imbricated bracts. Basal flowers female, fruits round-ovate and seedy. (Source: C.Y. Wu, China).*

undescribed species is the beautiful and attractive *Musa exotica* R. Valmayor, sp. nov. collected from the Cuc Phuong Forest Reservation and recently classified by Valmayor (2001). The banana explorers of Vietnam also collected wild specimens of *Ensete glaucum* (Roxb.) Cheesman. But the greatest find of the Vietnam banana collection project is Chuoi Canh, which is neither a *Musa* nor an *Ensete.* The authors have classified Chuoi Canh as a new species under *Musella*.

Botanical description of *Musella splendida* R. Valmayor & L. D. Danh, sp. nov.

General appearance of plant. Herbaceous and succulent with erect pseudostem formed by bases of clasping and persistent leafsheaths. Plants attain a height of 1.0 to 1.2 m at reproductive maturity. The primary inflorescence is terminal and erect. Suckers sprout from leaf bases at ground level (Figure 2). Old plants produce lateral inflorescences arising from leaf axils above ground.

Pseudostem and suckers. Pseudostem color green, shiny in young plants but turning dull at maturity with the appearance of waxy bloom. The plant is free suckering, many suckers sprout from the broad pseudostem base. As the mother plant grows older and the terminal inflorescence has set fruits, 2 to 5 lateral inflorescences branch out from the upper leaf bases clasping the pseudostem (Figure 3).

Petiole, midrib and leaf. Petiole solid green, no blotches but with few reddish purple dots close to the margins. Petiole margins curved inward and lined with red purple stripe (Figure 4). Midrib light green. Leaves large, elliptical and held upright on immature plants but

Figure 2. Musella splendida *bearing terminal inflorescence. Flower bud ovate, bracts with long, pointed tip loosely attached to the bud (Source: L.D. Danh, Vietnam).*

Figure 3. *Mature* Musella splendida *with lateral inflorescences branching out from pseudostem and suckers sprouting from leaf bases at ground level (Source: L.D. Danh, Vietnam).*

droop after flowering. Color of leaves darker green than that of the midrib, slightly glaucous with marked lateral veins. Leaf blade base symmetrical and pointed, apex acute. Leaf base-width ratio > 3.

Inflorescence and fruit bunch. Terminal inflorescence less than 30 cm long, peduncle short and massive, rachis obscure, completely covered by persistent dried bracts. Each bract subtends 2 to 6 flowers, hermaphrodite flowers are borne at the base of the inflores-

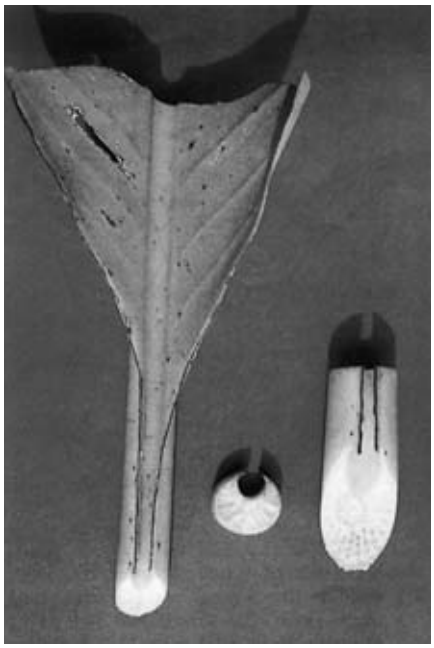

Figure 4. *Leaf blade base of* Musella splendida *with symmetrical, petiole margins curved inward with red stripe at edges (Source: L.D. Danh, Vietnam).*

Figure 5. *Inflorescence of* Musella splendida *showing bright yellow, persistent bracts that open simultaneously forming a magnificent crown (Source:L.D. Danh, Vietnam).*

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Figure 6. *Fruit cluster of* Musella splendida *pack below the male bud (Source: L.D. Danh, Vietnam).*

cence, male flowers at the top. Arrangement of flowers and fruits uniseriate. Male bud ovate, bracts glaucous, elongated tips loosely attached to the bud. Several bracts open at the same time forming a magnificent crown of the mature plant (Figure 5). Fruit bunch very compact, bracts bright yellow but upper margins lined with red stripe (Figure 6).

Flowers and fruits. Compound tepal and lobes of male flowers yellow. Free tepal opaque white, oval with folding tip. Free tepal same length as compound tepal. Anther color beige with pinkish tinge, filaments cream. Anthers are exserted at same level as compound tepal. Stigma same color as anthers (Figure 7). Ovary opaque white with 3 locules, each containing 2 rows of ovules. Fruits ovate, densely packed in single rows numbering 2 to 4 fruits per cluster. Dark green and pubescent at immature stage, turning yellow upon ripening. Fruits held perpendicular to the stalk by very short pedicel. Peel thick, adherent to the white, inedible pulp. Fruit seedless and parthenocarpic (Figure 8).

Original accession collected from the forest of northern Ha Giang province, Vietnam, on May 5, 1994 by Director Le Dinh Danh. Accession No. VN1-038; planting material, suckers; Important physiographic data: latitude, 22.49°north; longitude, 104.59° east; elevation, 118 m.; average rainfall, 24.30 mm; Average temperature, 22.7°C (max 40°C/July, min 1.5°C/Jan.); Soil, fertile forest soil with abundant moisture; parent material, limestone. Vast forests cover the northern mountain ranges of Vietnam bordering southern China.

Musella specimens that appear distinct from the type species, *M. lasiocarpa*, and from the newly described species, *M. splendida*, show variability in *Musella* (Figure 9). It is possible that with improved accessibility to and communication with China and Myanmar, additional species will be discovered.

Holotypus: Herbarium specimen held at Phu Ho Fruit Research Center, Phu Ninh, Phu Tho, Vietnam. Living accessions now growing in the National Banana Germplasm Collection at Phu Ho Fruit Research Center.

Diagnostic characters of *Musella splendida* in Latin and English

(In Latin). *Musella splendida* R. Valmayor & L.D. Danh sp. nov. differt magnopere a *Musella lasiocarpa* (A.R. Franchet) C.Y. Wu ex Li Hsi-wen statura plantae structura inflorescentiae et characterious floresque fructuum. Plantae *Musella splendidae* ampliores, 1.0- 1.2 m altae variantes, prae quam *Musella lasiocarpae* usque ad tantum 0.6 m. Folia uterque specierum ellipticae, in *Musella splendidis* plures triplo longiora quam latiora, in *Musella lasiocarpis* minores triplo. Alabastrum inflorescentiarum *Musella splendidae* ovatum sed aperiens ad apicum, quandoquidem apicibus acuminatis bractearum praecocibus discedentibus, ante complicarentes deorsum basibus, quamquam alabastrum *Musella lasiocarpae* deltiodeum bracteis valde imbricatis. Flores basales speciei novae hermaphroditi, sed *Musella lasiocarpae* feminei. Postremo, fructus *Musella splendidae* parthenocarpicus et exseminales, sed *Musella lasiocarpae* semina viabilia ferentes. Epitheton splendida elegantique magnificenti speciei novae selecta.

(In English). *Musella splendida* R. Valmayor & L.D. Danh sp. nov. is differentiated from *Musella lasiocarpa* (A.R. Frachet) C.Y. Wu ex Li Hsi-wen by major differences in plant stature, inflorescence structure, and characteristics of flowers and fruits. Plant of *Musella splendida* is larger, ranging from 1.0 to 1.2 m high compared with the smaller *Musella lasiocarpa*, which is less than 0.6 m tall. While the leaves of both species are elliptical, the leaf length-width ratio of the Vietnam specimen is >3 while that of China is <3. The inflorescence bud of *Musella splendida* is ovate but the apex is open as the long, pointed tips of individual bracts spread apart precociously, prior to folding down at the base. *Musella lasiocarpa* produces a deltoid inflorescence bud with tightly attached, markedly imbricated bracts. The basal flowers of the new species are hermaphrodite while those of the type species are female. Finally, the fruits of *Musella splendida* are seedless and parthenocarpic but those of *Musella lasiocarpa* bear viable seeds. The term *splendida* was selected to describe the elegance and magnificence of the new species.

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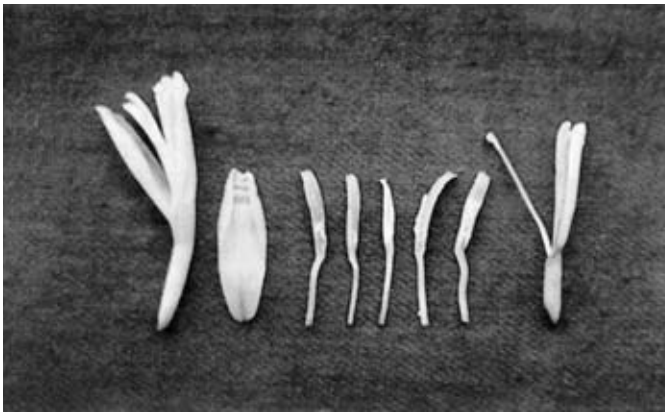

Figure 7. Musella splendida *male flower parts showing corrugated tip of free tepal and anthers with abundant pollen grains (Source: L.D. Danh, Vietnam).*

Figure 8. *Cross-section showing seedless, parthenocarpic fruits of* Musella splendida *(Source: L.D. Danh, Vietnam).*

Figure 9. *Interesting* Musella *specimens that look different from the type species,* Musella lasiocarpa*, and the new species,* Musella splendida *(Photographs: courtesy of M. Häkkinen).*

INIBAP/ASPNET, Philippines, for retrieval of indispensable information. ■

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Genetic diversity of East African Highland bananas using AFLP

*A.K. Tugume, G.W. Lubega and P.R. Rubaihayo**

Originally from Southeast Asia (Simmonds 1966, Valmayor *et al.* 1981), bananas are believed to have entered the East African highland region through multiple introductions between the first and sixth century AD (Price 1995). A wide range of unique varieties belonging to the East African Highland bananas (AAA-EA) now exist in the region, having evolved locally. The East African Highland region has been called a secondary centre of *Musa* diversity (Stover and Simmonds 1987, Swennen and Vuylsteke 1988), with Uganda showing the highest level of diversity of AAA-EA genotypes (Kyobe 1981, Rubaihayo and Mukasa 1993).

Knowing the degree of genetic relatedness between clones and the range of diversity present in *Musa* germplasm is important for conservation and the selection of parents for breeding programmes (Garwel and Jarret 1992, Ortiz *et al.* 1995, Lagoda *et al.* 1999). Morphological traits have been widely used in clone identification and taxonomic studies (Brewbaker and Umali 1956, Allen 1965, Stover and Simmonds 1987, Sebasigari 1990).

Karamura (1998) used 73 morphological traits to classify the East African Highland bananas of Uganda into five clone sets: *Mbidde* (Beer), *Musakala*, *Nakabululu*, *Nfuuka* and *Nakitembe*. However, the complexity of interactions between genes and the environment (Shanmugavelu *et al.* 1992) creates problems as elaborate field-testing is required for a classification to be effective (De Langhe 1990, Bhat *et al.* 1997, Oliviera *et al.* 2000, Valmayor *et al.* 2000). The narrower the genetic base, the less discriminating morphotaxonomy becomes (Jarret and Garwel 1995).

Molecular techniques have the potential of revealing stable genetic information on which to base classification. Amplified Fragment Length Polymorphism (AFLP) has been shown to be a powerful molecular tool (Donini e*t al.* 1997) capable of detecting genetic differences between related *Musa* accessions (Engelborghs *et al.* 1998) and closely related individuals (Jones *et al.* 1998). This paper reports the results of studies undertaken to assess the genetic relationships among East African Highland bananas using the AFLP technique.

Materials and methods

Young cigar leaf tissues from 115 East African Highland bananas were collected from the Uganda banana germplasm resource center at Kawanda Agricultural Research Institute, and Makerere University Agricultural Research Institute in Kabanyolo. The accessions were chosen on the basis of their expected low levels of polyphenols (Maliyakale 1992, Pich and Schubert 1993).

DNA was isolated from fresh leaf material (0.7 g) according to the protocol described by Vroh *et al.* (1996) but with some modifications. After the first chloroform extraction, a second extraction using 10% N-Cetyl-N,N,N-trimethylammonium bromide (CTAB), followed by repeated chloroform extractions, was added to ensure effective precipitation and elimination of proteins and carbohydrates (Rowland and Nguyen 1993). The composition of the CTAB buffer was modified by increasing polyvinylpyrrolidone (PVP-40) from 2% to 4%, and ß-Mercaptoethanol from 5% to 8%. The problem of polyphenols (Maliyakale 1992, Pich and Schubert 1993) was counteracted by raising the concentrations of PVP-40 (polyvinylpyrrolidone) and ß-Mercaptoethanol in the original CTAB buffer. The DNA yield was estimated by spectrophotometry in a *SmartSpect™ 3000 Version* 1.00.39 (BIORAD), as described by Linacero *et al.* (1998). Spectrophotometry and electrophoresis (Linacero *et al.* 1998) were used to assess the quality of DNA.

The molecular biology grade reagents for AFLP analysis were AFLP analysis system I kits (AFLP Core reagent kit and AFLP Starter primer kit) from Life Technologies (GIBCO BRL®). This system which has been designed for use in plants having genomes raging in size from 0.5 X 10^9 - 6 X 10^9 bp was used under license by Keygene N.V. Restriction digestion was carried out using 2.5 U of *Eco*R I and 2.5 U of *Mse* I restriction enzymes on 500 ng DNA as described in the AFLP Analysis system I manual. Assessment of the efficiency of digestion was carried out as recommended by Scott *et al.* (1998). Ligation of oligonucleotide adapters (*Eco*R I and *Mse* I adapters) was performed according to AFLP Analysis system I manual, and the adapters used (Table 1) were those described by Vos *et al.* (1995) for the restriction enzymes *Eco*R I and *Mse* I and were not phosphorylated.

Pre-selective PCR (Polymerase chain reaction) amplification of target sequences in DNA was performed as described by Vos *et al.* (1995) in a *PerKin Elmer® Model 2400 Thermocycler* using 2 pre-amplification primers without selective nucleotides:

1. *Eco*R I+0: 5'-GACTGCGTACCAATTC-3'
   and
2. Mse I+0: 5'-GATGAGTCCTGAGTAA-3'

Selective PCR amplification was performed using 2 ologinucleotide primers, one correspoding to *Eco*R I ends and the other to *Mse* I ends, each with 3 selective nucleotides (*Eco*R I+3 and Mse I+3).

Four selective primer pairs were used:

*Eco*RI+3
E1 5'-GACTGCGTACCAATTC**aac**-3'
E2 5'-GACTGCGTACCAATTC**acc**-3'
E3 5'-GACTGCGTACCAATTC**act**-3'
E4 5'-GACTGCGTACCAATTC**agc**-3
Mse I+3
M1 5'-ATGAGTCCTGAGTAA**ctt**-3'
M2 5'-GATGAGTCCTGAGTAA**caa**-3'
M3 5'-GATGAGTCCTGAGTAA**ctg**-3'
M4 5'-GATGAGTCCTGAGTAA**cag**-3'

One of the primers in each pair (*Eco*R I+3 primer) was radio-labelled with 2000 Ci/mmol [γ^{33}P]ATP (*Amersham Pharmacia Biotech*) using T_4 Polynucleotide Kinase by phosphorylating at the 5' end using the protocol of the AFLP Analysis system I instruction manual. The pre-selective PCR amplification products were diluted 100X with 1XTE buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA [Ethylene diamine tetrachloro acetic acid]) to be used for selective PCR amplification, and the reaction was a 36-cycle event performed in an *iCycler* (*BIO-RAD*), according to Vos *et al.* (1995).

Following selective PCR, reaction products were mixed with equal volumes (20 µl) of formamide loading dye (98% Formamide, 10 mM EDTA pH 8.0; 0.1% Bromophenol and 0.1% Xylene cyanol FF

as tracking dyes). The resulting mixtures were heated for 4 minutes at 95ºC and then quickly chilled on ice. Four microlitres of each sample were loaded on 0.4 mm 6% denaturing (sequencing) polyacrylamide gels. The gel matrix was prepared using 6% Acrylamide, 0.3% N,N'-Methylene bisacrylamide, 7.5 M Urea in 1XTBE buffer pH 8.0 (100 mM Tris, 90mM Boric acid, 1 mM EDTA). To 75 ml of the gel solution, 250 µl of freshly prepared 10% APS (ammonium persulphate) and 50 µl of TEMED (N,N,N'N'-Tetramethylene diamine) were added and the solution was mixed gently but quickly with a syringe. Electrophoresed was performed at 1800 V (constant), 37 mA, 65 W for 1 hour and 10 min, using 1XTBE buffer pH 8.0 as a running buffer. The gels were later fixed for 30 minutes in a fixing solution (5% glacial acetic acid, 4.8% ethanol) to drain off the urea and the blue dyes, dried and exposed to an X-ray film 35 X 43 cm (Biomax MR Kodak) for 48 hours at room temperature.

Selective PCR amplification products (amplified fragments) on the X-ray film were scored: '1' for presence and '0' for absence of a homologous fragment (band). Genetic distance data matrices were constructed using the method of Nei and Li (1979) and group average clustering were performed by the un-weighted pair-group method using arithmetic averages (UPGMA) (Sneath and Sokal 1973). The entire analysis and drawing of the phenogram were performed using TREECON Version 1.3b phylogenetic program for Window-based environment (Van de Peer and De Wachter 1994).

Results and discussion

The AFLP technique produced amplified fragments in the range of 20-350bp. The variation noted in the fragment size was attributed to the variation in the selective sequence of the *EcoR* I and *Mse* I primers (van Treuren 2001).

The results of UPGMA clustering of the 115 AAA-EA genotypes tested are shown in Figure 1. Low bootstrap values (0 – 85%) suggested absence of clusters and close genetic relationships among the cultivars. The majority of accessions were in the range of 0.1 – 0.4 Nei's genetic distance from each other, which also shows close genetic relatedness.

The majority of the AAA-EA accessions tested ended up in the same clone set identified by Karamura (1998) (*Musakala*, *Nfuuka*, *Nakitembe*, and *Nakabululu*) but some AAA-EA accessions fell into a different group (Table 1). With the exception of the beer clone set, which did not show up as a distinct group in our analysis, the clone sets proposed by Karamura (1998) were subclusters under the AFLP method. The unrooted analysis (Figure 2) further showed that each subcluster was subdivided into sub-subclusters.

Musakala, which the unrooted data analysis classified as the most distinct subcluster, was grouped into 4 sub-subclusters and separated from the other subclusters at 0.72 Nei's genetic distance (Figure 2). Karamura (1998) also reported *Musakala* to be the most distinct cluster and indicated that its accessions are characterized by uniquely giant, lax and long bunches and fingers.

Results also showed that *Nfuuka* was very closely related to *Nakitembe* and *Nakabululu*, as was also reported by Karamura (1998). Although *Nfuuka* was reported to be the most heterogeneous and largest cluster by Karamura (1998), it was the smallest subcluster in our analysis. The most distinguishing feature of *Nfuuka* subcluster is the ability of its accessions to alter phenotypes over time (Karamura 1998), hence its name, *Nfuuka*; which literally means "I am changing", "I am going to change", or " I keep changing". The natural structural rearrangements that frequently occur within and between banana chromosomes may make any attempts to develop realistic classification difficult (Faure *et al*. 1993). The accessions in the *Nfuuka* subcluster could be undergoing such a process that makes it able to alter phenotypes over time. The central topological position of *Nfuuka* subcluster on the unrooted phenogram (Figure 2) and the "keep changing" nature of *Nfuuka* reported by Karamura (1998), from which *Musakala*, *Nakitembe* and *Nakabululu* subclusters branch off (Figure 1) suggests that it could be responsible for the generation of other subcluster accessions.

Nakabululu and *Nakitembe* very closely related, according to our analysis. Karamura (1998) also reported that *Nakabululu* and *Nakitembe* were closely related, a closeness reflected by the early maturation of their accessions. Results showed that *Nakabululu* and *Nakitembe* were separated by negligible bootstrap support at the branch point and majority of accessions separated by less than 0.5 Nei's genetic distance from each other (Figure 1) indicating close genetic relationship. Although Karamura (1998) reported that *Musakala* and *Beer* were the two most distinct clusters, our analysis put *Nakabululu* and *Musakala* as the most distinct subclusters, grouping them at the opposite ends of the phenogram (Figure 1). This is further reflected by the extreme bunch compactness and short fingers of the *Nakabululu* accessions versus the large luxuriant bunches with long fingers of the *Musakala* accessions (Karamura 1998).

Our analysis did not put the beer bananas in a distinct cluster but mixed them with the cooking types. The beer bananas were found to be versions of the cooking bananas but different at a locus (loci) responsible for astringent sap (synthesis of tannins and anthocyanins).

The obligate vegetative reproduction of bananas have led them to maintain the features which accompanied their ancestors when introduced in the region (Simmonds 1966). Somatic mutations, with preferential cultivation of mutants by man, has resulted in the low levels of genetic diversity seen in the germplasm. According to Karamura (1998), a wide diversity of the physical features, climates and social backgrounds of the East African region have played a significant role in the diversification of different clones. It is possible that the high frequency of translocations and (retro)transposons and irregularities in meiosis and methylation (Lagoda *et al*. 1999) have been responsible for the narrow genetic diversity observed in East African Highland bananas in Uganda.

The low levels of DNA diversity in this study contrasts with the high level of morphological diversity present in these genotypes reported by Karamura (1998), probably due to the influence of genotype X environment interaction on morphotaxonomic techniques (Shanmugavelu *et al*. 1992). The discordance between the AFLP classification and the morphological classification could also be explained by the primers used in our analysis.

The selective sequence of a primer is one of the determining factors of the multiplex ratio (the number of different loci that can be simultaneously analyzed per experiment) (Bryene *et al*. 1997). Results indicated (data not shown) that individual primer combinations could not reveal enough polymorphism upon which to draw conclusions, because some parts of the genome were left unscreened for polymorphism (van Treuren 2001).

Acknowledgments

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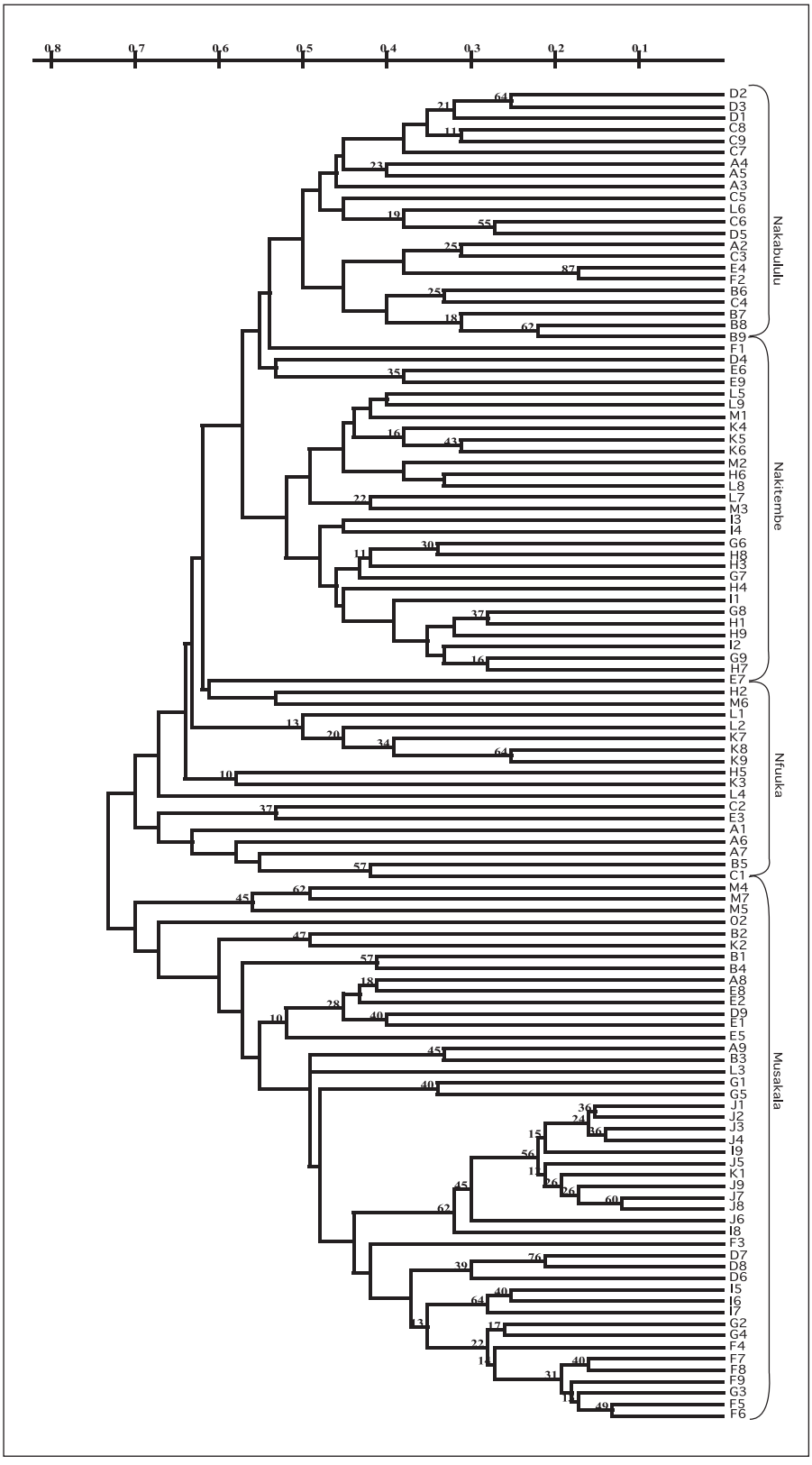
Table 1. Classification of 115 East African Highland banana accessions based on rooted and unrooted analyses of AFLP data, and compared to a classification based on morphological characters (Karamura and Pickersgill, 1999).

AFLP classification Code*	Name	Morphological classification
***Subcluster* Musakala**		
Sub subcluster 1 (MS1)		
F6	Mutiliti	*Musakala*
F5	Oruhuna	*Beer*
G3	Kifuba	*Nfuuka*
F9	Kisaabo	*Nfuuka*
F8	Lwandungu	*Nfuuka*
F7	Shombobureku	*Beer*
F4	Entazidukwa	*Nfuuka*
G4	Bareka	*No record*
G2	Mwanga	*Beer*
I7	Mugisu-agenda	*Musakala*
I6	Kisansa	*Musakala*
I5	Rwabakongo	*Musakala*
D6	Enjogabakazi	*Musakala*
D8	Mukazi-alanda	*Musakala*
D7	Nalweunzika	*Musakala*
F3	Enyabakazi	*Musakala*
I8	Mpologoma	*Musakala*
J6	Bitambi	*Nfuuka*
Sub subcluster 2 (MS2)		
J8	Enzirabushera	*Nfuuka*
J7	Ndibwabalangira	*Nfuuka*
J9	Luwata	*Musakala*
K1	Mayovu	*Nfuuka*
J5	Enjagata	*Musakala*
I9	Nandigobe	*Nakitembe*
J4	Bikowekowe	*Nakitembe*
J3	Enyarutere	*Nakitembe*
J2	Nakibinyi	*Nfuuka*
J1	Kigerekyanjovu	*No record*
Sub subcluster 3 (MS3)		
G5	Muvubo	*Musakala*
G1	Nalugolima	*Musakala*
L3	Tulatwogere	*Nfuuka*
B3	Lwezinga	*Nfuuka*
A9	Keitaluganda	*Beer*
E5	Enzirabahima	*Nfuuka*
E1	Kifuba	*Nfuuka*
D9	Enyeru	*Nfuuka*
E2	Namafura	*Nfuuka*
E8	Kasitaza	*Nfuuka*
A8	Likhako	*Nfuuka*
Sub subcluster 4 (MS4)		
B4	Enyoya	*Musakala*
B1	Mudwale	*Beer*
K2	Lumenyamagali	*Musakala*
B2	Namunwe	*Musakala*
D2	Kabusi	*No record*
M5	Musakala	*Musakala*
M7	Bandagyeya	*Musakala*
M4	Nalukira	*Beer*
***Subcluster* Nfuuka**		
Sub subcluster 1 (NF1)		
C1	Namadhi	*Beer*
B5	Nasala	*Nakitembe*
A7	Lisandalo	*Nakitembe*
A6	Nambi	*Nfuuka*
A1	Nyamashari	*Nfuuka*
E3	Njeriadet	*Nfuuka*
C2	Nabusa	*Nfuuka*
L4	Nfuuka	*Nfuuka*
K3	Namwezi	*Nfuuka*
H5	Atwalira-Nyina	*Nfuuka*
Sub subcluster 2 (NF2)		
K9	Entukura	*Beer*
K8	Nakayonga	*Nakabululu*
K7	Katalimbwambuzi	*Beer*
L2	Nante	*Nfuuka*
L1	Siira white	*Nakitembe*
M6	Siira red	*Nfuuka*
H2	Nassaba	*Nfuuka*
E7	Kulwoni	*Nfuuka*
***Subcluster* Nakitembe**		
Sub subcluster 1 (NT1)		
H7	Nakitembe	*Nakitembe*
G9	Entaragaza	*Nakitembe*
I2	Enkonera	*Nfuuka*
H9	Mbwazirume	*Nakitembe*
H1	Salalugazi	*Nakitembe*
G8	Entundu	*Beer*
I1	Imbululu	*Beer*
H4	Enkara	*Beer*
G7	Luvuta	*Nakitembe*
H3	Kibidebide	*No record*
H8	Kibagampera	*Beer*
G6	Nakaangu	*Nakitembe*
Sub subcluster 2 (NT2)		
I4	Ekirama	*No record*
I3	Nakawere	*Nfuuka*
M3	Nalwera	*Nakitembe*
L7	Kafunze	*Nakabululu*
L8	Nakakongo	*No record*
H6	Mende	*Beer*
M2	Entanga	*Beer*
K6	Nakyetengu	*Nakitembe*
K5	Lwefusa	*Nfuuka*
K4	Namaliga	*Nakitembe*
M1	Bagandeseza	*Beer*
L9	Bifusi	*Nakabululu*
L5	Engumba	*Beer*
***Subcluster* Nakabululu**		
Sub subcluster 1 (NB1)		
E9	Bwara	*Beer*
E6	Tereza	*Nfuuka*
D4	Namulondo	*Nakitembe*
F1	Nakinyika	*Nfuuka*
Sub subcluster 2 (NB2)		
B9	Endembezi	*Beer*
B8	Enyambo	*Nfuuka*
B7	Namesti	*Beer*
C4	Kazirakwe	*Nakabululu*
B6	Wekanga	*Nakabululu*
F2	Kibuzi	*Nakabululu*
E4	Nakhaki	*Nfuuka*
C3	Nambogo	*Nfuuka*
A2	Nakasabira	*Nfuuka*
Sub subcluster 3 (NB3)		
D5	Namamuka	*Nfuuka*
C6	Nkobe	*Nfuuka*
L6	Ensasa	*Beer*
C5	Namunyere	*Nakabululu*
A3	Keitabunyonyi	*Nakabululu*
A5	Nalusi	*Beer*
A4	Ensika	*No record*
C7	Engambani	*Beer*
C9	Kabucuragye	*Nakabululu*
C8	Enshenyuka	*Beer*
D1	Butobe	*Nakabululu*
D3	Nakibuule	*No record*

*Codes are for identification purposes only.

Figure 1. *Phenogram of 115 East African Highland banana accessions from Uganda using Nei's genetic distance on AFLP data. The scaled bar on top of the phenogram refers to Nei's genetic distance and the numbers in the phenogram are bootstrap support values. The codes at the base of the phenogram refer to the accessions tested, which are presented in Table 1.*



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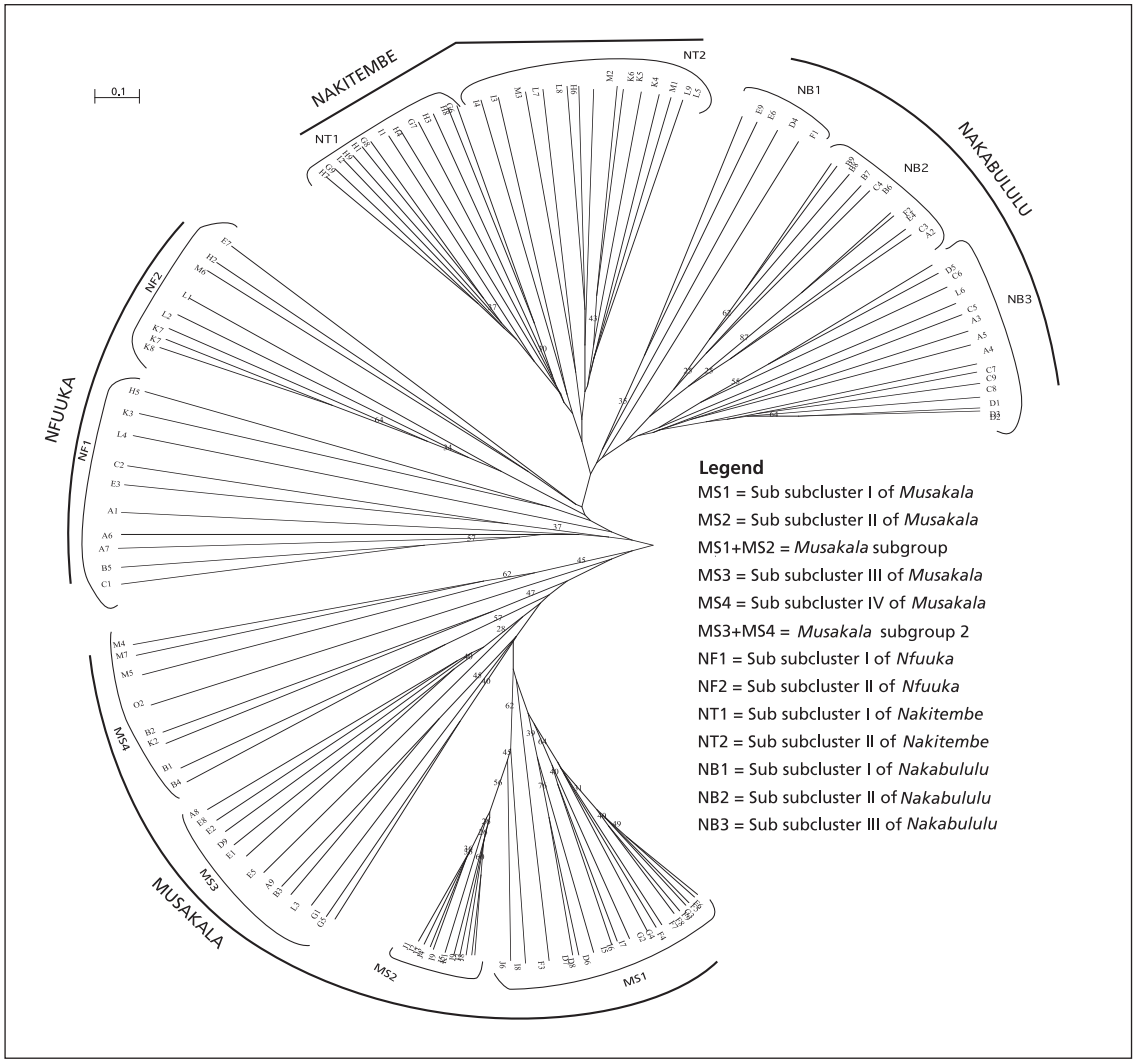

Figure 2. *Unrooted phenogram of 115 East African Highland banana accessions from Uganda using pooled AFLP data. Labels at branch points are bootstrap support values, and the bar on upper left hand corner of the phenogram is Nei's genetic distance scale. The codes refer to the accessions tested, which are presented in Table 1.*

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Identification of plant parasitic nematodes of plantain 'Dominico hartón' (*Musa* AAB Simmonds), 'Africa', 'FHIA-20' and 'FHIA-21' in Colombia

O.A. Guzmán-Piedrahita and J. Castaño-Zapata

In Colombia, about 400 000 hectares are planted with plantain and have a production of about 2 970 000 tonnes of which 96% is consumed locally and the remainder exported (Merchán 1996, Arcila and Torres 1998). Plantain is a basic foodstuff in the country and consumption is the third most important after potato and milk (Grisales-López and Lescot 1999), making it essential to the family shopping basket because of the nutritional qualities. Its cultivation is also a source of work and of important economic resources (Cruz *et al.* 1990).

The most important pests and diseases of plantain are leaf spot diseases (*Mycosphaerella* spp.), the banana borer weevil (*Cosmopolites sordidus)* that affects the corm, and plant parasitic nematodes that affect the roots and corm. The latter have necessitated the development of genetic, chemical, biological and cultural control strategies to reduce damage (Montiel *et al.* 1997).

Parasitic nematodes damage the roots and corm of plantain directly, and reduce yield, result in fewer and smaller leaves, reduce fruit weight, and induce plant toppling and rotting of the root system (Montiel *et al.* 1997). Yield loss is estimated at 20% (Saser and Freckman 1987 cited by Marín 1997) but may be up to 80% (Sarah 1989 cited by Marín 1997).

In the Musaceae, 146 species of nematode, distributed in 43 genera, are parasitic on or associated with the genus *Musa*. The most damaging and widely distributed of the plant nematodes are the migratory endoparasitic nematodes *Radopholus similis* and *Pratylenchus coffeae*, and the semiendoparasite *Helicotylenchus multicinctus* (Araya 1995). The sedentary endoparasitic nematode *Meloidogyne* and the semiendoparasitic sedentary nematode *Rotylenchus reniformis* are of less importance (Gowen and Quénéhervé 1990, Araya 1995).

R. similis (borer nematode) is the most economically important in most regions where banana and plantain are cultivated. Root symptoms comprise lesions, approximately 10 cm in length, reddish brown at first then black. Later the lesion becomes larger and forms cavities in the cortex, and the vascular tissue becomes exposed to invasion by secondary microorganisms, resulting in death of the root in the part behind the lesion. When infection is severe, the rhizome becomes necrotic hence the name "black head" (Ashby 1915 cited by Román 1978). Therefore the vegetative cycle is prolonged, and the size and numbers of leaves, and plant development are reduced. This reduces bunch weight and the productive life of the plantation, and increases plant toppling (Gowen and Quénéhervé 1990).

Eight species of the genus *Pratylenchus* are reported to attack Musaceae although only two are widely distributed and recognized as damaging: *P. coffeae* and *P. goodeyi* (Thorne 1961, Gowen and Quénéhervé 1990). *P. coffeae* (lesion nematode) was reported in roots of plantain in Granada and described as *Tylenchus musicola* by Cobb in 1919 and, unlike *P. goodeyi*, appears to be widely distributed throughout the world (Gowen and Quénéhervé 1990). Root symptoms comprise extensive black or purple necrosis of the epidermal and cortical tissues, often accompanied by secondary decay and splitting of the root (Gowen and Quénéhervé 1990).

H. multicinctus (spiral nematode) is, after *R. similis*, probably the most common and widely distributed of the nematodes in banana and plantain plantations throughout the world. Symptoms comprise superficial red lesions in the root epidermis and cortex (Román 1978, Gowen and Quénéhervé 1990).

Meloidogyne spp. (root knot nematodes) are found in the roots of banana and plantain wherever these crops are grown (De Waele and Davide 1998, Figueroa 1990). The first symptoms are the presence of swelling and galls in the primary roots and, to a lesser extent, in secondary and tertiary roots resulting in secondary symptoms such as yellowing of the aerial plant parts, narrowing of leaves, slowing of plant growth and reduced productivity, with losses up to 57%. These nematodes have a wide host range, especially the dicotyledonous plants that are often found in areas where Musaceae are cultivated (De Waele and Davide 1998).

The plantain 'Dominico hartón' belongs to the group *Musa* (AAB) subgroup plantain type 'Horn'. Elsewhere in Latin America it is known as 'Macho x Hembra', 'Maricongo' or 'Bastard'. It is relatively unstable which, in agreement with the planting altitude, shows the effect of genotype-environment interaction on the physiology of the plant and bunch. The cultivar is susceptible to *R. similis* and is considered as intermediate between 'Dominico' and 'Hartón' (Belalcázar 1991).

The cultivar 'Africa' is a selection of the African clone 'Mbouroukou' of 'Hartón' type, that adapts well from sea level to 1700 m, and a bunch weight of up to 26 kg and a fruit quality similar to that of 'Dominico hartón'; the cycle is about 10 months depending on fertilization, which allows maintenance of one or two functional leaves until harvest (www.corpoica.org.co 2000).

The hybrid 'FHIA-20' (AAAB) is resistant to Fusarium wilt (Panama disease), black leaf streak disease and Sigatoka disease, but is highly susceptible to *R. similis*; this hybrid is an alternative to the plantain 'Cuerno'. The high yield (20-30 kg without rachis) and excellent quality makes it preferred for consumption fresh, or cooked unripe or mature. Productivity can be two to three times higher than in 'Cuerno' (www.fhia.hn/banano.htm 1998).

The hybrid 'FHIA-21' (AAAB) is resistant to Fusarium wilt, black leaf streak disease and Sigatoka disease, is high yielding (22–27 kg without rachis) and of excellent quality. Hence this cultivar is also preferentially placed for fresh consumption, or cooked unripe or mature. The material is susceptible to *R. similis* and is cultivated by small-scale farmers and co-operatives in Honduras, Nicaragua and Ecuador, with excellent results. Given similar conditions, productivity can be two to three times greater than in 'Cuerno' plantain (www.fhia.hn/banano.htm 1998).

Because of the damage caused by nematodes to the roots and corm, and the lack of information available on their incidence in the municipality of Palestina

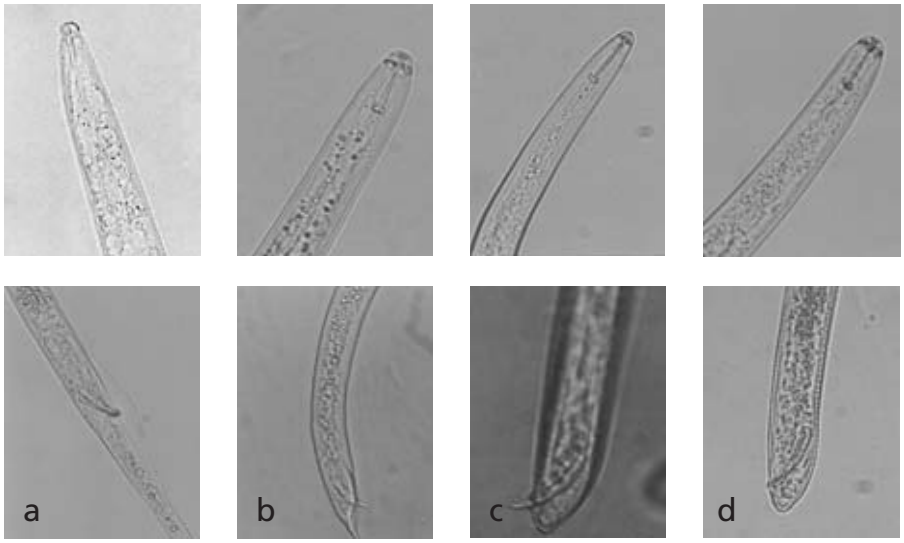

Figure 1. *Head and tail regions of males: a.* Radopholus similis, *b.* Pratylenchus coffeae, *c.* Helicotylenchus multicinctus, *d. Larva at stage J 4 of* Meloidogyne *spp.*

(Caldas, Colombia) the aim of the present study was to identify the genera and populations of parasitic nematodes that interfere with the development and function of the root system of plantain 'Dominico hartón', 'Africa', 'FHIA-20' and 'FHIA-21' at the Montelindo farm.

Materials and methods

The Montelindo farm, *Universidad de Caldas*, is situated in Santagueda, municipality of Palestina, department of Caldas. The farm is at an altitude of 1010 meters above sea level, and has a mean annual temperature of 22.8ºC and a rainfall of 2200 mm per year. The soil originates from fine-grained volcanic andesitic ash, classified as typic dystrandept, with good natural fertility and franco-sandy texture (Salazar and Duque 1994).

Plants of 'Africa', 'FHIA-20' and 'FHIA-21' were micropropagated in a tissue culture laboratory, of the *Departamento de Fitotecnía de la Universidad de Caldas*. 'Dominico hartón' was obtained as corms of about 1 kg in weight, from the Chagualito farm, municipality of Chinchiná, Caldas. Plants were 12 months old at the time of sampling and were planted 25 (5 x 5) per plot, spaced 3 x 2 m apart and surrounded with plantain 'Dominico hartón' at an equal spacing. Plots were arranged at random. Prior to planting, each plot was amended with 1 kg of ash-based mixture, 13 g of Furadan 3 GR (carbofuran), 10 g of MgO and 15 g of borax. Fertilizer was applied every four months making a total of three applications (200 g per plant), the first with NH_4NO_3 + KCl (1:1), the second with 15-4-23-4 'cumba' fertilizer and the third with NH_4SO_4 + KCl (1:1). Fungicides and nematicides were not applied. Suckers were thinned to two per plant, leaves were stripped (folded, dry leaves removed by cutting upwards at the leaf base) and weeded every 4 months with a scythe in the pathways and manually at each planting site.

Soil and root samples (200 g) were removed from the sucker less than 2 m in height from plants that had flowered not more than 8 days previously (Araya *et al.* 1995, Araya and Chaves 1997). Samples were removed with a rectangular shovel (13 x 50 cm width x length,) at a distance of 25 cm from the plant base from a 13 x 13 cm-hole and 30 cm-deep, in front of the sucker (Araya *et al.* 1995, Cabrales 1995). Soil and roots were collected in plastic bags, labelled and transferred to the plant pathology laboratory, *Departamento de Fitotecnía de la Universidad de Caldas*, Colombia for analysis.

Roots were washed in tap water for 3 min, the surfaces squeezed dry, and separated into functional (live) or non-functional (necrotic and dead) roots. Then, 25 g of functional roots were weighed (Scale: *Analytical Plus*, Ohaus, model AP210S), cut transversely with scissors into 1 cm pieces and homogenized (Araya *et al.* 1995). Extraction was by the blender, sugar centrifugal-flotation procedure (Castaño-Zapata *et al.* 1997, Araya *et al.* 1995) as follows: roots were placed in a blender (*Osterizer;* model 565-15) which was filled up to 1000 mL with tap water and set, first at low speed then at high speed, both for 30 seconds. The liquid was passed through No. 35, 100 and 400 (0.5, 0.150, 0.038 mm) sieves. The No. 35 and 100 sieves were washed for 2 min, and the contents transferred to a beaker and filled up to 100 mL with tap water. Next, the root-water mixture was homogenized with a glass rod for 30 seconds and emptied into centrifuge tubes balanced separately (3800 rpm for 5 min). The supernatant was discarded and the tubes filled with a sugar solution (500 g of sugar in 1 L of water) and centrifuged at 3800 rpm for 5 min; the supernatant was transferred to a N° 400 sieve, washed with distilled water to remove the sugar solution, transferred to a 100 ml beaker and made up to 20 mL with tap water. The suspension was homogenized with a glass rod for 10 seconds and 5 mL aliquots transferred to a reading chamber (Petri dish divided into 8 squares) and counted with the aid of a stereomicroscope (Nikon). The 5 mL sample was returned to the beaker, the contents shaken and counting repeated three times. The mean population of nematodes/mL and the total nematode population per sample were calculated. Twenty nematodes were transferred with a dissecting needle to a slide with a drop of water, covered and examined under a compound microscope (Nikon) with a 40X objective for identification using taxonomic keys (Luc *et al.* 1990, Thorne 1961, Taylor 1968, Román 1978, Castaño-Zapata and Salazar 1998). Population means were subjected to analysis of variance and differences between plant materials evaluated using the MSTAT – C statistical programme University of Michigan (Bricker 1993).

Results and discussion

Two genera and two species of plant parasitic nematode were identified: *Pratylenchus* spp. *Meloidogyne* spp., *H. multicinctus* and *R. similis* (Figures 1 and 2). With the exception of *R. similis* for 'Africa' and 'FHIA-20' plantain, the remaining nematodes affected the plantain materials that were studied. Populations were in the order: *Meloidogyne* spp., *R. similis, H. multicinctus* and *Pratylenchus* spp. (Table 1), substantiating the habitat of these species and the conditions for obligate parasites (Thorne 1968, Román 1978).

The largest *R. similis* populations were recorded in the roots of 'FHIA-21' and 'Dominico hartón' (Table 1), which explains the high percentage of toppling of these genotypes on the experimental site, thus greatly reducing their productivity.

The low populations of *Pratylenchus* spp. in the Santágueda region substantiated the observations of Araya *et al.* (1993) that this nematode is more frequent in cool climates.

Table 1. Number of nematodes per 100 g of roots identified in four cultivars of plantain.

Cultivar	*R. similis*		*Pratylenchus* spp.		*H. multicinctus*		*Meloidogyne* spp.	
	Soil	Roots	Soil	Roots	Soil	Roots	Soil	Roots
Dominico hartón	412*	2605	412	650	204	1166	412	710
Africa	0	0	285	797	3136	1940	3704	3220
FHIA-20	0	0	860	675	312	2032	2580	3819
FHIA-21	1168	3135	1752	1486	584	3096	2336	4040

*Mean of three replicates.

R. similis was not recorded in 'Africa' and 'FHIA-20' whereas *Meloidogyne* populations were relatively high (Table 1). The absence of *R. similis*, the species that most limits production of banana and plantain throughout the world, in these two genotypes is a factor implicated in the high yield of these materials under the conditions at the Montelindo farm. It should be noted that 'FHIA-20' is susceptible to this nematode. Its absence in soil and root samples can be attributed to the absence of inoculum, probably because the plots were previously established with Bermuda grass [*Cynodon dactylon* (L.) Pers.], or to inherited resistance from 'SH-3437' which is resistant to plant nematodes (Binks and Gowen 1996).

In the coffee producing zones of the Colombian Andes, 231 000 ha of plantain are cultivated, with a production of 1 650 000 tonnes per year, equivalent to 67% of the national production (Rodríguez and Rodríguez 2001). *R. similis* is a serious threat to the exploitation of plantain, and also predisposes plants to the effects of other pathogens such as fungi, bacteria and viruses. Therefore it is necessary to monitor populations and implement integrated management in commercial plantations. ■

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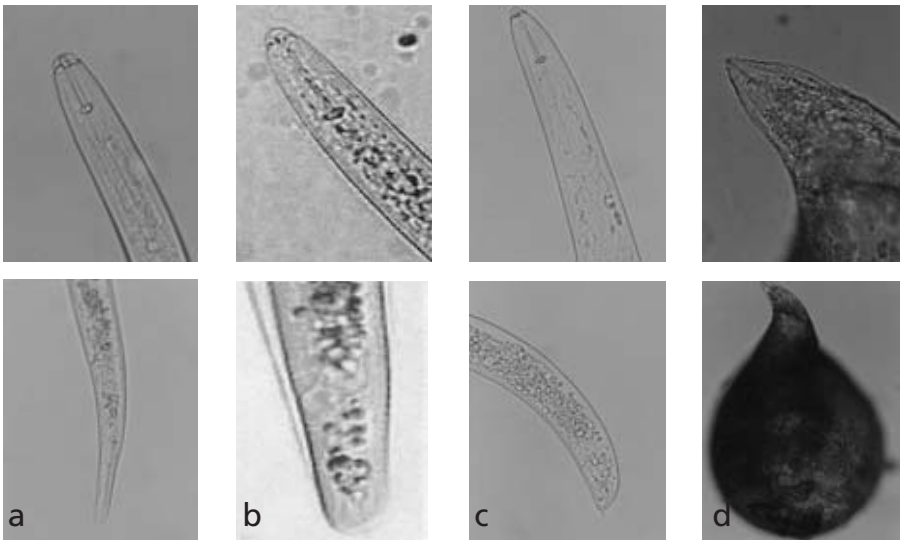

Figure 2. *Head and tail regions of females of the species and genera identified: a.* Radopholus similis, *b.* Pratylenchus coffeae, *c.* Helicotylenchus multicinctus, *d. Head and entire female of* Meloidogyne *spp.*

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Comparison of temporary and permanent immersion systems for the *in vitro* culture of banana

K. Matsumoto and A.K.C. Brandão

Micropropagated plantlets are increasingly used for the commercial cultivation of bananas because the plant material is clean, easy to manipulate and makes it possible to synchronize harvest. However, the existing micropropagation techniques using banana meristem and shoot tips (Cronauer and Krikorian 1984, Gupta 1986, Wong 1986, Vuylsteke 1989) are labour-intensive and require large laboratory space. Consequently, the production cost of micropropagated plantlets is very high, making small-scale farmers reluctant to use these superior plantlets.

The production cost can be reduced by resorting to large-scale *in vitro* cultures using bioreactors. Various types of bioreactors have been adapted for plant micropropagation (Levin *et al.* 1988, Akita *et al.* 1994, Lim *et al.* 1998, Lorenzo *et al.* 1998). Temporary immersion systems were successfully used in the micropropagation of bananas of the AAA (Alvard *et al.* 1993, Lemos *et al.* 2000) and AAAB groups (Daquinta *et al.* 2000). The objective of this study was to compare a temporary immersion system with a permanent immersion system and a conventional culture system using a Brazilian AAB group cultivar.

Materials and methods

In vitro plantlets of the cultivar 'Maçã' (AAB) induced from shoot tips were maintained on Murashige and Skoog (MS) medium supplemented with 2 µM indole-3-acetic acid (IAA), 2 µM 6-benzylaminopurine (BA) and 2 g/L Phytagel (Sigma Co.) in 300 mL flasks. The cultures were maintained in a controlled environment room at 27 ± 2°C and 16 hr photoperiod with light intensity of 33 µmol m^{-2} s^{-1}, using cool white fluorescent tubes. Each 10 mm long *in vitro* explant, including meristem, was longitudinally cut in two and transferred to the bioreactors.

The temporary immersion system was set up as reported by Escalona *et al.* (1999). Ten-litre flasks were used to keep the explants and five litre flasks the culture medium (Figure 1). One hundred and thirty explants (65 meristems) were transferred to each bioreactor with 2000 mL of medium (one meristem per 30 mL of medium). The explants were immersed in medium for four minutes every four hours. The culture medium was composed of MS medium with 22 µM BA and 100 mg/L of Claforam for microbial control.

In the permanent immersion system, the same volume of medium and size of flasks as the ones in the temporary immersion system were used. Sterilized filtered air provided by an air pump (600 mL/s) was injected every hour for five seconds. Thirty meristems were put in the bioreactor (one meristem per 66 mL of medium).

In the conventional culture system, the explants were cultured on semi-solid MS medium solidified by 0.2 g/L Phytagel (Sigma Co.). In each 300 mL flask, four explants were inoculated to 50 mL of medium (Magenta Box®, Sigma Co.) (one meristem per 25 mL of medium). Ten flasks were used, for a total of 40 explants.

The cultures were maintained in a controlled environment room (27 ± 2°C, under cool white fluorescent light of 33 µmol m^{-2} s^{-1}, 16 hr photoperiod). After four weeks, the number of shoots, their height, and the fresh weight of the shoots and buds were noted. The data were analysed using a one-way analysis of variance (ANOVA) followed Duncan's multiple range tests at p=0.05.

Results and discussion

Using 10 litre flasks increases the risk of microbial contamination but they allow for the explants to be maintained in the same flask until the plantlets have roots long enough for acclimatization (Figure 2). We are still not sure of the effectiveness of the antibiotic (Claforam) used here to reduce the risk of contamination, but we recommend such a precautionary treatment.

The temporary immersion system and permanent immersion system respectively produced 3.7 and 12 times more plant material than the conventional culture system (Table 1). The shoots produced in the conventional culture system were also significantly shorter than the shoots produced in the other two systems. However, the number of shoots produced in the conventional culture system was

Figure 1. *In the temporary immersion system, the bioreactor was a 10 litre flask and a 5 litre flask was used as reservoir for the medium.*

Figure 2. *Two-month-old* in vitro *plantlets produced in the temporary immersion system ready for acclimatization.*

not significantly different than the one in the temporary immersion system. This is because we only counted the shoots that were at least 5 mm long. Had the threshold been 10 mm, the number of shoots in the conventional culture system would have been lower.

The permanent immersion system produced the tallest shoots but fewer of them. The permanent immersion system is also simpler and easier to install than the temporary immersion system, but it produces high levels of vitrification and meristematic rhizome growth that are not favourable for micropropagation (Vuylsteke 1989).

In banana tissue culture, the goal is to produce a maximum number of shoots long enough for rooting. In this respect, the temporary immersion system gave the best overall results among the systems tested, a finding which is consistent with previous studies (Daquinta *et al.* 2000, Lemos *et al.* 2000).

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Table 1. Fresh weight of shoots and buds, number of shoots and shoot height of explants after four weeks of culture (mean ± standard error).

Culture system	Fresh weight of shoots and buds* (g)	Number of shoots per explant**	Shoot height (mm)
Temporary immersion system	5.38 ± 0.34 b	2.83 ± 0.22 b	34.6 ± 0.30 b
	(n=37)	(n=37)	(n=105)
Permanent immersion system	17.94 ± 5.01 c	1.42 ± 0.59 a	50.6 ± 0.80 c
	(n=26)	(n=26)	(n=36)
Conventional culture system	1.45 ± 0.16 a	2.67 ± 0.14 b	14.4 ± 0.15 a
	(n=37)	(n=37)	(n=98)

* The explants initially weighed about 200 mg each.

** Shoots of 5 mm or more.

Values followed by a different letter are significantly different at p<0.05 (Duncan's multiple range test).

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The effect of soil bulk density on root and overall plant development in six banana varieties

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Root development and distribution are not only genotype-dependent (Kasperbauer 1990, McMichael 1990, Klepper 1992, Zobel, 1992), they are also affected by the environment (Jung 1978, Kasperbauer 1990). Soil structure, availability of nutrients and water, temperature and drainage can interact with the genetic make-up of the plant (Hamblin 1985, Box 1996, Aguilar *et al*. 2000). For example, mechanical impedance was shown to reduce the root elongation rate which was related to an increase in soil bulk density or penetrometer pressure (Voorhees *et al*. 1975, Babalola and Lal 1977, Maurya and Lal 1979, Panayiotopoulos *et al*. 1994).

Tillage reduces the mechanical impedance and the bulk density of the soil (Russell 1977). For dessert bananas, Robinson (1996) found that soils which had been ploughed below 50 cm had more roots and healthier ones. The effect of reducing the bulk density of the soil has not been studied in a wide range of *Musa* spp. genotypes. The objective of this study was to assess the effect of tilling on the root and shoot characteristics of several *Musa* spp. genotypes.

Materials and methods

This study was carried out at the IITA High Rainfall station at Onne (4°42' N, 7°10' E, 5 masl), in southeastern Nigeria, where the soil is derived from coastal sediments and is a deep and freely drained Typic Paleudult/Haplic Acrisol (FAO/ISRIC/ISSS 1998). It belongs to the coarse-loamy, siliceous isohyperthermic family. The chemical and physical properties of the topsoil in the experimental fields were assessed (Table 1). The average annual rainfall is 2400 mm distributed monomodally from February until November (Ortiz *et al*. 1997). Six genotypes belonging to four *Musa* spp. groups were assessed: the dessert banana 'Valery' (AAA), the plantains 'Agbagba' and 'Obino l'ewai' (AAB), the cooking banana 'Fougamou' (ABB) and the tetraploid plantain hybrids 'TMPx 548-9' and 'TMPx 1658-4'. These hybrids were obtained by crossing 'Obino l'ewai' with 'Calcutta 4' (*M. acuminata* ssp. *burmannicoides*) and 'Pisang lilin' (*M. acuminata* ssp. *malaccensis*), respectively. The planting material consisted of sword suckers (i.e. lateral shoots with lanceolated leaves) which were pared and planted according to Swennen (1990).

Two experiments were carried out. In the first experiment, land preparation was done manually, using hoes, to avoid soil disturbance. In the second experiment, on the contrary, the soil was harrowed and ploughed to a depth of 30 cm, using a tractor, one week prior to planting. Both experimental fields had been under grass fallow for more than five years prior to planting. The first experiment was planted in June 1996 and the second one in August 1998. Despite the two-year interval in planting dates, the soils' physical and chemical characteristics were similar at the time of planting (Table 1), the two fields being only three meters apart. In addition, the rainfall was abundant during both trial periods: 805 mm during the period June to August 1996 and 960 mm during the period August to October 1998.

The field layout in each trial was a randomized complete block design with two replications of two plants per genotype. Plant spacing was 2 m x 2 m. The plants were grown under monocropping system and no mulch was applied. The experimental area was treated with the nematicide Nemacur (a.i. fenamiphos) at a rate of 15 g/plant (three treatments per year) to reduce nematode infestation. Fertilizer was applied in the form of muriate of potassium (60% K) at a rate of 600 g/plant annually, and urea (47% N) at a rate of 300 g/plant annually, spread equally over six applications during the rainy season. The fungicide Bayfidan (a.i. triadimenol) was applied three times per year at a rate of 3.6 mL/plant to control black leaf streak disease caused by *Mycosphaerella fijiensis* Morelet.

Plants in both experiments were excavated 12 weeks after planting, and shoot and root characteristics were assessed. Aerial growth data measured on each plant included plant height and leaf area. Leaf area was calculated according to Obiefuna and Ndubizu (1979). Corm fresh weight was also measured. Root characteristics included the number of adventitious roots, or cord roots, and root dry weight. The cord root length was measured using the line intersect method (Newman 1966, Tennant 1975). This method consists in scattering cord roots on a 3 cm by 3 cm grid and counting the number of interaction points. The number of interaction points were multiplied by a conversion factor of 2.3571, appropriate for the grid size used.

Results and discussion

There was a significant effect of tillage on all root and shoot characteristics (Table 2). Plants in the tilled field grew better than those in the non-tilled field (Figures 1 and 2). Tillage reduced the bulk density of the soil by 26% (from 1.64±0.06 g/cm^3 to 1.21±0.02 g/cm^3) at a depth of 5 cm, and by 27% (from 1.62±0.07 g/cm^3 to 1.18±0.12 g/cm^3) at a depth of 20 cm.

The positive effect of a reduced soil bulk density on root growth had been demonstrated under mulched conditions. Avnimelech (1986) reported that there was a clear change in the soil structure due to the application of organic matter. Soil aggregates change from compacted dense to friable porous aggregates allowing root penetration, water and solute diffusion in and out of the aggregates and thus improving the properties of the soil as a growing medium.

For *Musa* spp., Swennen (1984) reported an increased root ramification under mulched conditions. In addition, under shifting cultivation, plantain is established during the first year after land clearing when soil organic matter content is maximal and soil bulk density low. Similarly, the vigorous growth of plantains and bananas in backyard systems (Nweke *et al*. 1988) may be partially attributed to a high organic matter content and an associated low soil bulk density.

For the East African Highland banana cv. 'Mbwazirume' (AAA), McIntyre *et al*. (2000) reported that mulched treatments were more productive in terms of both above and below ground biomass than bare soil treatments. Lower soil bulk density and faster water recharge at depth in mulched treatments indicated that mulching increased soil porosity and improved infiltration.

Salau *et al.* (1992) attributed the enhanced vegetative growth and bunch yield of a mulched crop of plantain to increased soil porosity and soil infiltration capacity, among others. The positive effect of increased soil porosity on root growth and development has also been demonstrated for dessert bananas (Sioussaram 1968, Champion and Sioussaram 1970, Delvaux and Guyot 1989, Robinson 1996) and for the highland banana cv. Mbwazirume (McIntyre *et al.* 2000).

Our study showed that reducing the bulk density of the soil by tilling increased shoot growth, which was associated with enhanced root development. This stresses the importance for bananas of adopting cultural practices that reduce the bulk density of the soil.

Table 1. Chemical and physical properties of the topsoil in both experimental fields at the time of planting.

	Tilled		Non-tilled	
Depth (cm)	**0-15**	**15-30**	**0-15**	**15-30**
Sand %	80	76	79	73
Silt %	6	4	7	5
Clay %	14	20	14	22
PH H_2O (1:1)	4.2	4.2	4.0	4.3
Org C %	1.38	0.87	1.09	0.8
Kjel N %	0.116	0.079	0.105	0.065
C/N ratio	12	11	10	12
Bray-I P (ppm)	60.4	47.7	60.1	62
Exch Ca (cmol/kg)	0.9	0.4	0.2	0.2
Exch Mg (cmol/kg)	0.4	0.1	0.2	0.1
Exch K (cmol/kg)	0.1	0.03	0.1	0.1
Exch Na (cmol/kg)	0.1	0.1	0.4	0.5
Exch Mn (cmol/kg)	0.01	0	0.02	0.01
Exch Acid (cmol/kg)	3.0	3.0	2.7	3.0
Exch.Al (cmol/kg)	2.0	2.0	2.0	1.7
ECEC (cmol/kg)	4.5	3.6	2.5	2.8

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Table 2. Mean squares and significance for different traits.

Source of variation	df	Shoot and root characteristics						
		LA	**PH**	**PC**	**CW**	**DR**	**NR**	**LR**
Land preparation	1	2527959649***	31682***	2067***	21817971***	14938***	26654***	11306501***
Genotype	5	24826299	384	20	703096	71	1235	162260
Replication	1	26267980	14	0.3	88714	214	348	166471
Residual	39	56895732	362	33	1142765	247	576	337071

LA: leaf area, PH: plant height, PC: plant circumference, CW: corm fresh weight, DR: root dry weight, NR: number of cord roots, LR: cord root length.
*** Significant at P<0.001.

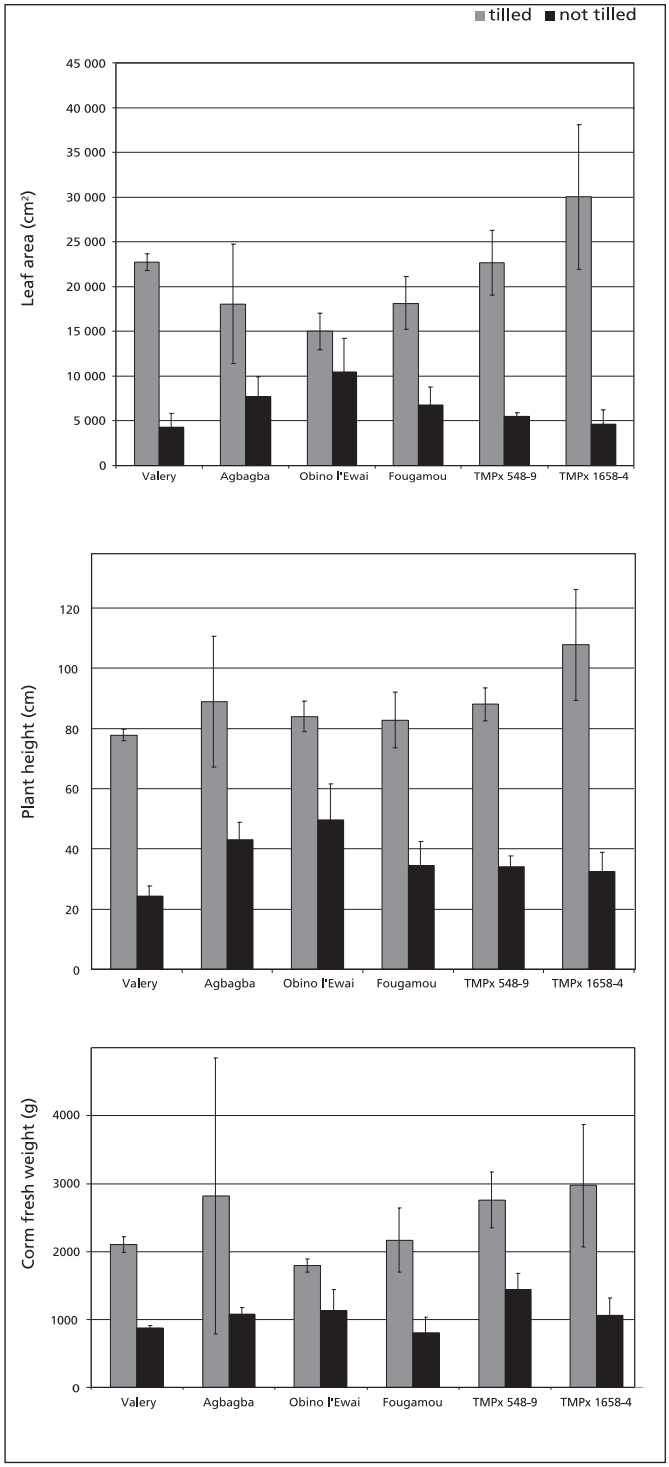

Figure 1. *Leaf area, plant height and corm fresh weight of six different banana varieties growing in a tilled and a non-tilled soil.*

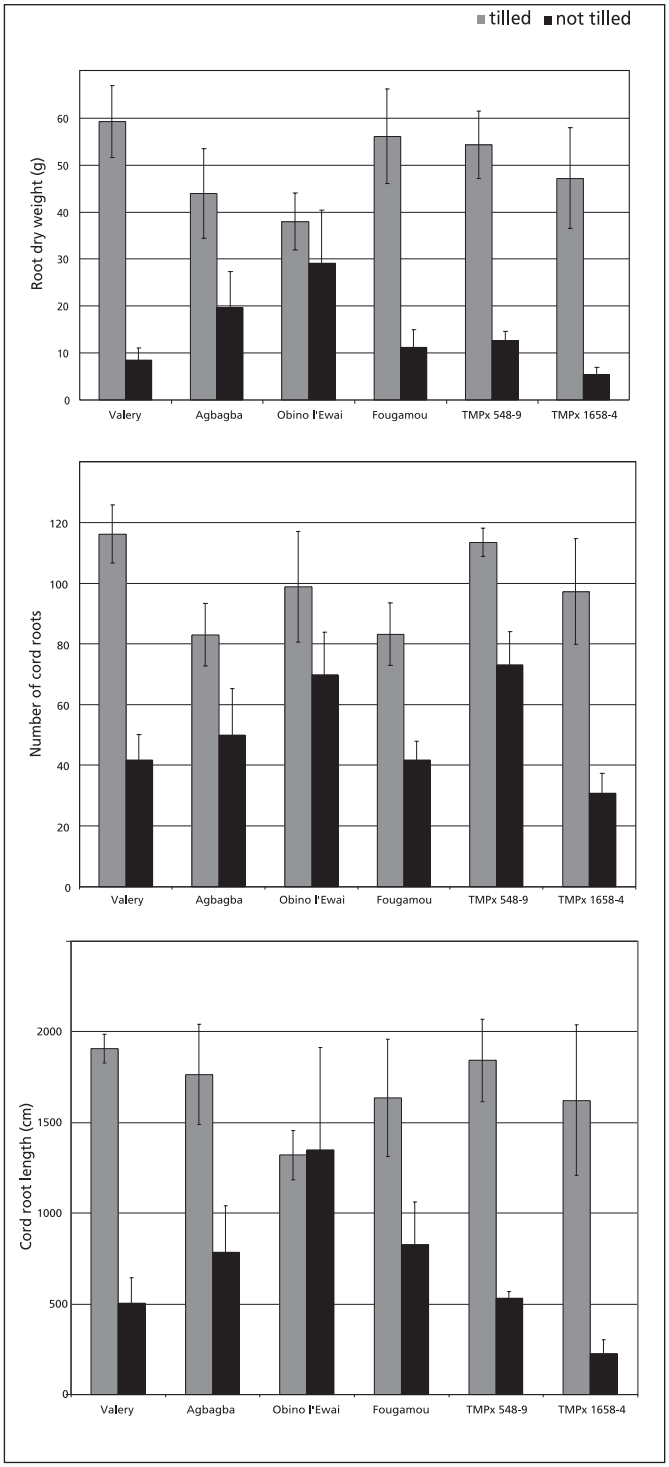

Figure 2. *Root dry weight, number of cord roots and cord root length of six different banana varieties growing in a tilled and a non-tilled soil.*

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Use of different types of inoculum of *Mycosphaerella fijiensis* Morelet to evaluate the behaviour of two cultivars of banana in greenhouse conditions

M. Leiva Mora, M.A. Dita Rodríguez, Y. Alvarado Capó, M. Acosta Suárez, L. García and I. Bermúdez

The presence of *Mycosphaerella fijiensis* Morelet in the main *Musa* producing regions has lead to a decline in yields. The development of methods for early selection requires that symptoms be expressed in controlled conditions to ensure the greatest chances of success in genetic improvement programmes. The purpose of the present work was to evaluate the behaviour of two cultivars of banana with different levels of resistance to *M. fijiensis*, and to select the best type of inoculum for selection in greenhouse conditions.

Vitroplants which had been acclimatized for eight weeks, and had an average size of 20 cm and four active leaves, were evaluated in a greenhouse. The plants represented the cultivars 'Grande naine' and 'FHIA-18', which show different levels of resistance to *M. fijiensis*.

A pathogenic isolate of *M. fijiensis*, CC-ibp-1, was used to prepare an inoculum of 10^6 cfu/mL, which was mixed with 1% gelatin and applied by means of a small brush to the tissue of the lower surface of the first three open leaves.

For comparison, fragments of diseased leaves at stage 6 on Fouré's scale (1982) were collected in natural conditions, cut into pieces 4x4 cm and placed at the base of each plant as a source of infection.

Incubation for the first 72 hours was at 95-100% relative humidity (RH). From the fourth day RH was maintained above 50% during the day, and at saturation (100%) during the night.

Symptom severity	Symptoms	Level of resistance
0	A predominance of healthy leaves	Highly resistant
1	A predominance of leaves with small red dotted lesions	
2	A predominance of leaves with regular or irregular reddish spots on their lower surface	Partially resistant
3	A predominance of leaves with regular or irregular dark red spots on their upper surface	
4	A predominance of leaves with black spots (elliptical or circular), chlorotic margins and a watery halo containing green sectors	Susceptible
5	A predominance of leaves with grey-centred black spots, which can hang down from the pseudostem	

The time to the appearance of the first symptoms (incubation period) and the severity of symptoms were evaluated for each cultivar with the respective level of resistance, according to the scale proposed by Alvarado *et al*. (2002).

The results showed that it was possible to observe disease symptoms on both cultivars of banana in greenhouse conditions using different types of *M. fijiensis* inoculum. Symptoms comprised elliptical or circular spots and were similar to those seen on "orejones" (cabbage-like) suckers in natural conditions.

The presence of these symptoms can be explained by the limited differentiation of foliar tissue of the vitroplants. Differentiation of the leaf veins acted as barriers to the spread of *M. fijiensis* hyphae (Mourichon *et al*. 2000).

The incubation period was very similar in both cultivars irrespective of the type of inoculum (Table 1).

The mycelial homogenate gave the best results: preparation was simple and inoculum concentration could be calculated and adjusted before use. Mycelial homogenates also avoided the presence of saprophytic microorganisms or other pathogens, and they could be used for isolates that did not produce conidia, and at any time of the year. In contrast, fragments of diseased leaves could only be collected in dry periods or when there was little rainfall (Jones 1995, Balint-Kurti *et al*. 2001).

From the fourth week of incubation, the severity of attack was greater in 'Grande naine' than in 'FHIA-18' irrespective of inoculum type. From the sixth week, the difference became more marked because symptom development was slower in 'FHIA-18' (Table 1).

Similar results were obtained by Romero and Sutton (1997) who studied the response of 'FHIA-01' and 'FHIA-02' to an inoculum comprising suspensions of conidia. The authors indicated that, although the mechanism of resistance of these genotypes was still not known, a low density of stomata, an increase in extracuticular deposits of leaf wax and the production of phytoalexins, suberin or lignin have been suggested as possible mechanisms associated with the partial resistance in these hybrids.

The susceptibility of 'Grande naine' with respect to the partial resistance

Table 1. Incubation period and mean symptom severity of two banana cultivars inoculated with different types of *M. fijiensis* inoculum.

Type of Inoculum	Incubation period (days)		Mean symptom severity							
	'Grande naine'	'FHIA-18'	'Grande naine'				'FHIA-18'			
			W4	W6	W7	W8	W4	W6	W7	W8
Fragments of diseased leaves	14	15	2	3	5	5	1	2	3	3
Mycelial homogenate	14	16	2	3	4	5	1	2	3	3

W4… = Week 4…

shown by 'FHIA-18' in greenhouse conditions was found to coincide with the response of both cultivars in natural conditions.

These results confirm the possibility of evaluating the early response of different cultivars of *Musa* at an early stage against the causal agent of black leaf streak disease. ■

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Short communication | **Measurement of leaf area**

A new factor for estimating total leaf area in banana

N. Kumar, V. Krishnamoorthy, L. Nalina and K. Soorianathasundharam

Leaf area is one of the parameters used to determine a plant's photosynthesis potential. Watson (1947) applied the concept of leaf area to measure the productive potential of field crops. He defined a leaf area index as the area of green leaf per unit area of land.

Leaf area can be measured by destructive methods, but the non-destructive method of linear measurement was found to be simple, inexpensive and accurate (Yeboach *et al.* 1984). For bananas, Murray (1960) suggested a 'K' factor of 0.80 to be multiplied by the length and breadth of the leaf. This method gives the area of the leaf in question but not the total leaf area of the plant, which is of more interest to researchers. One way to obtain the latter is to estimate the area of each leaf, using Murray's method, and to

Table 1. Total leaf area.

	Column number							
	1	2	3	4	5	6	7	8
Plant No.	Number of leaves per plant	Estimated area of third leaf (m^2)	Estimated total leaf area (m^2) (1 x 2)	Actual total leaf area (m^2)	Individual K_2 factors (4/3)	Predicted total leaf area (m^2) (2 x 1 x K_2)	Difference between predicted and actual (6-4)	Difference (%)
1	15	1.689	25.3320	16.663	0.658	16.775	0.112	0.667
2	15	1.715	25.718	16.155	0.628	17.030	0.875	5.139
3	18	1.593	28.674	18.581	0.648	18.988	0.407	2.144
4	16	1.741	27.854	18.469	0.663	18.445	-0.024	-0.128
5	15	1.766	26.496	16.824	0.635	17.546	0.722	4.115
6	16	1.705	27.286	17.031	0.624	18.069	1.039	5.747
7	14	1.389	19.449	13.41	0.690	12.880	-0.538	-4.176
8	14	1.777	24.878	16.699	0.671	16.474	-0.225	-1.365
9	15	1.669	25.041	15.897	0.635	16.582	0.685	4.131
10	14	0.990	13.866	9.152	0.660	9.182	0.030	0.321
11	13	0.966	12.563	8.156	0.649	8.319	0.163	1.960
12	12	0.841	10.090	6.584	0.653	6.681	0.097	1.455
13	12	0.875	10.502	6.845	0.652	6.955	0.110	1.574
14	12	1.131	13.574	8.951	0.659	8.989	0.038	0.420
15	14	0.287	4.017	2.825	0.703	2.660	-0.166	-6.226
16	13	0.446	5.795	4.003	0.691	3.838	-0.165	-4.294
17	13	0.469	6.101	4.124	0.676	4.040	-0.084	-2.067
18	13	0.579	7.532	5.213	0.692	4.988	-0.225	-4.515
19	14	0.348	4.869	3.254	0.668	3.224	-0.030	-0.922
20	14	0.584	8.172	5.269	0.645	5.411	0.143	2.633
21	15	0.422	6.330	4.256	0.672	4.192	-0.065	-1.541
22	13	0.509	6.620	4.622	0.698	4.384	-0.238	-5.430
23	14	0.389	5.445	3.626	0.666	3.605	-0.020	-0.565
24	13	0.421	5.476	3.626	0.662	3.626	0.000	0.004
25	14	0.401	5.615	3.689	0.657	3.718	0.029	0.791
Mean	14.04	0.99	14.29	9.357	0.662	9.378	0.107	-0.005
t-test						0.950		
r		0.986*	0.999**	0.999	-0.536	0.999 **	0.533	0.536

* Statistically significant at probability 0.05

** Statistically significant at probability 0.01.

add them up, but this is cumbersome and time consuming. Instead, many workers just measure the leaf area of the third leaf, using Murray's method, and multiply by the total number of leaves, but this is unsatisfactory as leaf size varies during development. The objective of this study was to estimate a second constant 'K_2' to obtain a better estimate of the total leaf area of the plant.

Materials and methods

Twenty-five plants of banana comprising 15 'Robusta' (AAA), 5 'Rasthali' (AAB) and 5 'Karpooravalli' (ABB) were removed at various stages of growth: 3 months after planting, 5 months after planting and at shooting. The number of leaves were counted and the area of the third leaf was estimated using the formula A=L x B x K, where A= estimated leaf area, L= leaf length, B= leaf breadth and K= 0.8. The estimated area of the third leaf was multiplied by the number of leaves to obtain the estimated total leaf area. The actual total leaf area of each plant was measured in a conveyer belt leaf area meter LICOR Model 3001.

An individual K_2 value for each plant was obtained by dividing the actual total area by the estimated total area. Then the mean of all individual K_2 values was calculated to obtain the value of 0.662 for the constant K_2. This value was used to calculate the predicted total leaf area which was then compared to the actual total leaf area. A t-test was carried out and the difference between actual leaf area and predicted area was worked out.

Results and discussion

The difference between the estimated total area using the third leaf method and the actual and predicted total areas was large for all 25 plants (Table 1). A t-test performed on the estimated and predicted leaf area showed that they were significantly different.

A positive and significant correlation was obtained between actual and predicted leaf area (r=0.999), suggesting that a value of 0.662 for K_2 is a good way to estimate *in situ* the total leaf area of a banana plant.

Based on this result, we propose to measure the total leaf area of a banana plant by counting the total number of leaves (N), measuring the length (L) and breadth (B) of the third leaf from the top and calculating the total leaf area (TLA) as follows: TLA=L x B x 0.80 x N x 0.662. ■

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Short communication | **Cultural practices**

Postharvest characteristics of 'Rasthali' bananas grown under different polyethylene covers

K.A. Shanmugasundaram and R.S. Azhakia Manavalan

In the tropics and sub-tropics, bunch covers are commonly used to protect bananas from rust thrips (Smith 1947, Simmonds 1969, Sivakumar and Mohanasundaram 1971, Wardlaw 1972). We tested transparent and non-transparent polyethylene covers to compare their effectiveness in eliminating blemishes and to examine their effect on the post-harvest characteristics of the 'Rasthali' banana.

Materials and methods

Five 100 cm x 60 cm polyethylene materials were tested:

- 50 gauge high-density transparent white polyethylene (HDT White)
- 150 gauge low-density transparent white polyethylene (LDT White)
- 150 gauge reprocessed transparent blue polyethylene (RT Blue)
- 200 gauge reprocessed non-transparent blue polyethylene (RNT Blue)
- 200 gauge reprocessed non-transparent black polyethylene (RNT Black).

Bunches of 'Rasthali' bananas were covered when the first hand opened and 0.4% ventilation was provided (Stover and Simmonds 1987). Control bunches were not covered. Three of the five plants per treatment were maintained separately to record the number of days to maturity. The covered bunches were harvested when the angularity of the fruits was about to disappear. The bunches were dehanded with a curved knife to minimize damage during transport. The hands were brought to the laboratory and kept under ambient atmosphere for observation. The second hand was used for quality analysis (Dadzie and Orchard 1997). Fruit characteristics, quality, mechanical damage and thrip damage were statistically analysed using a randomized block design.

Result and discussion

The results show that covers reduced thrip damage (rusty spots) by preventing thrips from reaching the flowers (Table 1). These results agree with the findings of Bhaktavatsalam *et al*. (1968), Hinz *et al*. (1999) and Jager and Daneel (1997).

Hard lump formation was nearly absent on the fruits kept under a transparent cover (Table 1). This is attributed to the increase in temperature caused by the polyethylene cover as higher temperatures increase the conversion of starch into sugar (Ganry 1975, Reddy 1989). Interestingly, hard lump formation was higher in bunches grown under non-transparent covers than in the control ones because light is necessary for normal fruit development and ripening. Stover and Simmonds (1987) also reported that the use of pigmented bags had not improved quality.

At harvest, the skin colour of the covered fruits was exceptionally uniform: attractive and clear, without any patches or blemishes (Figure 1). The fruits grown under transparent covers were more attractive, with a uniform dark green, than the fruits covered with non-transparent polyethylene. This is because the filtered sunlight

allowed the skin to synthesise chlorophyll (Choudhury *et al*. 1996, Heenan 1973, Stover and Simmonds 1987).

The fruits covered with reprocessed non-transparent black polyethylene were glossy white when unripe, as a result of the almost complete filtration of sunlight (98.3%). Devoid of chlorophyll, the fruits remained glossy white. The ripe fruits exhibited a dull white colour and the skin was more vulnerable to handling, contrary to the findings of Pandey (1994) and Nayak (1999). Though non-transparent covers reduced blemishes, they cannot be recommended because they aggravated hard lump formation, whereas transparent covers reduced both.

Although bunch covers tend to reduce mechanical damage (Parmar and Chundawat 1984), in our tests, the fruits grown under the reprocessed non-transparent black covers recorded the highest mechanical damage (Table 1). This is probably due to the fragility of the resulting white skin.

The green life of the covered bunches was one or two days longer than the one of the control fruits. This is probably because the decision to harvest was based on the disappearance of the angles. Since the fruits under the covers were not exposed to the environment, they lost less water (Johns and Scott 1989) and being filled with water, the fruits gave the appearance of being fully mature. The covered fruits indeed had a higher moisture content than the uncovered fruits which hardened well, but filled up later and ripened earlier than the covered fruits. Others also reported a longer green life for covered fruits (Johns and Scott 1989, Choudhury *et al*. 1996, Nayak 1999), whereas Parmar and Chundawat (1984) reported a reduction in green life using a blue polyethylene cover over 'Basarai' banana.

The fruits grown under a cover lost more weight after being harvested than the control ones (Table 2), probably as a consequence of going from a higher to a lower humidity environment. Parmar and Chundawat (1984), on the contrary, recorded a lower weight loss in covered fruits.

Figure 1. *Fruits grown under a high-density transparent polyethylene.*

With regard to quality, the covered fruits, except for those under non-transparent covers, had more total soluble solids than the control ones, probably because the higher temperature under the cover favoured the conversion of starch into sugars. Parmar and Chundawat (1984) also reported similar findings. The reduction in the content of total soluble solids in fruits grown under non-transparent covers might be due to the higher moisture content of these fruits and the presence of hard lumps (lack of conversion of starch into sugars). The sugar to acid ratio of the covered fruits was also higher than the one of the control fruits.

Though the covers effectively prevented thrip damage, their use adds to the production cost. Among the polyethylene covers used, the high-density transparent white polyethylene was the least expensive, followed by the reprocessed transparent blue polyethylene and the low-density transparent white polyethylene. However, the bunches covered with the high-density transparent white polyethylene were vulnerable to wind damage because of the thinness of the material, making it unsuitable in wind prone areas.

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Table 1. The influence of different polyethylene covers on the postharvest fruit characteristics of 'Rasthali' bananas.

Type of cover	Thrip damage (%)	Mechanical damage (%)	Fruit peel colour at harvest	Presence of hard lumps*	Green life (days)	Total soluble solids (º Brix)	Sugar to acid ratio
HDT White	3.92 ^{a}	5.60 ^{b}	Dark green	1.14	5.60 ^{b}	26.36 ^{e}	56.73 ^{a}
LDT White	4.29 ^{a}	6.20 ^{b}	Dark green	1.46	5.80 ^{b}	25.04 ^{b}	55.58 ^{a}
RT Blue	5.36 ^{a}	2.80 ^{a}	Green	1.68	5.60 ^{b}	26.24 ^{de}	58.68 ^{ab}
RNT Blue	4.29 ^{a}	1.60 ^{a}	Pale green	3.42	5.20 ^{b}	25.36 ^{c}	66.74 ^{bc}
RNT Black	10.42 ^{a}	10.40 ^{c}	Glossy white	3.66	5.40 ^{b}	24.36 ^{a}	70.08 ^{c}
Control	21.5 ^{b}	7.40 ^{b}	Dark green	3.10	4.40 ^{a}	26.04 ^{d}	54.52 ^{a}

Mean values followed by the same letter are not significantly different at 5% level.

*Index for the presence of hard lumps (Nil: 1, Low: 2, Medium: 3, High: 4, Very high: 5).

Table 2. Physiological weight loss (%) in fruits of Rasthali bananas grown under different polyethylene covers.

	Days after harvesting						
Type of cover	1st	2nd	3rd	4th	5th	6th	7th
HDT White	1.51 a	3.18 d	4.38 b	5.56 bc	7.03 b	8.57 b	11.33 b
LDT White	1.74 b	3.70 e	5.27 d	6.80 d	8.33 de	9.88 c	12.26 c
RT Blue	1.47 a	2.76 b	3.72 a	4.81 a	6.34 a	7.87 a	10.51 a
RNT Blue	1.50 a	3.17 d	4.84 c	6.95 d	8.53 e	10.35 d	12.5 b
RNT Black	1.44 a	2.94 c	4.24 b	5.83 c	8.11 cd	10.47 d	12.76 c
Control	1.42 a	2.53 a	3.66 a	5.06 ab	7.80 c	9.77 c	12.39 c
Mean	1.52	3.05	4.35	5.84	7.69	9.48	11.96

Mean values followed by the same letter are not significantly different at 5%. level.

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Thesis

Analysis of the diversity within the fungal genus *Cylindrocladium*: application to the phenotypic, molecular and pathogenic characterization of isolates from the banana rhizosphere

PhD thesis submitted in September 2002 to the *Ecole nationale supérieure d'agronomie de Montpellier*, France

Jean-Michel Risède

Filamentous fungi of the genus *Cylindrocladium* have been known for many years to be partly responsible for root necrotic lesions that induce root breakage and toppling in banana cropping systems. Until now the implicated taxa have never been typed probably because species are difficult to identify in this genus. Consequently a multicriteria diversity study aimed at characterizing these species and developing a molecular diagnostic tool of *Cylindrocladium* species was undertaken.

Phenotypic and biological analysis of *Cylindrocladium* isolates from the banana rhizosphere yielded a structure based on fives morphotypes with different geographical distribution. Isolates from MT1, MT3, MT4 and MT5 morphotypes could not be clearly identified by their phenotypic traits because of their similarity with different complexes of morphologically similar species. MT2 isolates seemed to be conspecific with the species *C. spathiphylli*.

Analysis of ribosomal spacer DNA polymorphism pointed out the conserved nature of the ITS region in the genus *Cylindrocladium* whereas the IGS region displayed polymorphism that can be used for discrimination of species. CAPS on the amplified intergenic spacer represents a rapid and reliable molecular diagnostic tool of *Cylindrocladium* species that can easily be transferred in banana producing zones. It revealed that MT2 and MT5 isolates are respectively conspecific with *C. spathiphylli* and *C. gracile* while MT3 and MT4 are related to the species *C. scoparium* and *C. floridanum sensu lato*. Despite their atypical phenotype, MT1 isolates were shown to be closely related to *C. gracile*. RAPD markers revealed that they only have 60% genetic similarity with this species. Taking into account their overall characteristics, they were recognized as a related undescribed species called *C. macrogracile*.

Pathogenicity evaluation of these five taxa towards banana yielded that *C. spathiphylli* and *C. macrogracile* as respectively highly and moderately aggressive on the variety 'Grande naine' (AAA). The three other species showed weak to no pathogenicity. Inoculation of six different banana genotypes revealed significant differences in susceptibility, but no true differential interactions between isolates and banana genotypes.

RAPD markers also indicated a low genetic variation within the species *C. spathiphylli* and *C. macrogracile*, suggesting their possible clonal propa-

gation in banana cropping systems. Within *C. spathiphylli* a partition according to host separated isolates originating from bananas to those collected on heliconias. This partition was illustrated at different levels by spacer rDNA polymorphism, RAPD markers and pathogenicity on bananas.

This study offers many challenging research perspectives among which the possibility to develop from the IGS region species-specific PCR primers that could further simplify identification of these fungi and favour the development of detection tests using plant or soil samples. ■

Study of the genetic mechanisms implicated in the expression in banana plants of pathogenic EPRV sequences in the course of interspecific genetic crosses

PhD thesis submitted in July 2002 to the *Ecole nationale supérieure d'agronomie de Montpellier*, France

Fabrice Lheureux

Interspecific hybrids have, in the past few years, been shown to express symptom of banana streak disease (BSD) that could not have originated from external sources of banana streak virus (BSV). It was hypothesised that infections arose instead through a complex recombination pattern from viral sequences integrated into the *Musa* genome called BSV-OL endogenous pararetrovirus sequences (EPRVs). The aim of our study was to search for the genetic factor that activates EPRV expression during genetic cross hybridisation of two interspecific crosses: PKW (BB) x IDN 110 4x (AAAA) and P. batu (BB) x P. pipit 4x (AAAA).

The study of the two F1 progeny showed Mendelian segregation of the disease with half of the hybrids containing virus. The observation of BSV strain Ol EPRV segregation occurs in a homozygous state in the *Musa balbisiana* genome only. Ten AFLP markers were selected in female *M. balbisiana* parents. The segregation analysis resulted in a genetic map of the locus responsible for disease expression, banana streak virus expressed locus (BEL), to be proposed. These data indicate that a genetic mechanism is involved in BSV expression and suggest that a monogenic allelic system confers the role of carrier to the *M. balbisiana* parent.

Two previously unidentified BSV strains in addition to BSV-Ol, have been detected in diseased hybrids: BSV-Imové (BSV-Im) and BSV-Gold Finger (BSV-GF) strains. The BSV-Ol and BSV-Im strains appeared in almost all diseased hybrids. In contrast, the BSV-GF strain was detected in only 49 percent of the diseased hybrids tested. The presence of BSV-GF and BSV-Im EPRVs among parental and progeny lines, shows that the *M. balbisiana* genome contains at least two other pathogenic BSV EPRVs. Genetic analysis resulting from AFLP results showed that the expression of BSV-O1 and BSV-Im EPRVs depends on the same genetic factor, the BEL locus. Although BSV-GF is not genetically linked to the BEL locus, it seems to be subordinated to it. So, the discovery of new BSV EPRVs linked to the BEL locus suggests that BEL differs in nature from BSV EPRVs.

Finally, we have investigated the regulation of EPRVs by investigating the "healthy" behaviour of *M. balbisiana* parents. A resistance to EPRVs expression has been demonstrated even when these parents are inoculated with BSV. Propositions as for the origin of the integration mechanism of BSV are discussed. ■

Impact of banana growing on the environment. Influence of growing systems on erosion, water balance and nutrient losses on a volcanic soil in Martinique: the case of a rust-brown halloysitic soil

PhD thesis submitted in June 2001 to the *Ecole nationale supérieure d'agronomie de Montpellier*, France

Bounmanh Khamsouk

Intensively practised in the French West Indies, banana growing requires a large quantity of fertilisers and pesticides. Localized near watercourses and inhabited areas, this intensive monoculture could therefore have repercussions on the environment since the island environment provides conditions, such as high annual rainfall and hilly topography, which favour the degradation of cultivated land (by water erosion) and chemical pollution of the runoff or drainage water leaving the banana plantations. In view of the demand for soil conservation measures and respect of the environ-

ment, the setting up of a field study would enable the evaluation of this intensive monoculture on the environment whilst satisfying the objectives of 1) studying runoff and erosion on a volcanic soil in a tropical island climate and 2) estimating chemical losses by erosion and drainage.

With this aim we set up ten experimental plots (100-200 m^2) to test some of the banana growing systems recommended by CIRAD-FLHOR (rotation with pineapple or sugar cane using cultural techniques of varying intensity) on slopes of 10%, 25% and 40% and subjected to risks of runoff and erosion. We also placed five lysimeters under banana plants to establish the water balance and to estimate the chemical losses due to leaching. In 1999 and 2000, the ten plots received different treatments: "bare soil", "mulched sugar cane", "established banana plantation", "mulched pineapple grown on a flat surface", and "mechanised, ridged, pineapple". At the same time we carried out laboratory tests for stability and structure, and field tests, with simulated rainfall, to assess the behaviour of the soil and to gain a better understanding of runoff and erosion.

The results obtained in 1999 and 2000 were very similar, despite the difference in annual rainfall, and they show the effect of treatments. On "bare soil" the loss of soil (85 to 165 t/ha/yr) increases with the slope (11% to 40%) whereas runoff decreases (184 to 87 mm). These results show that non-selective erosion changes as the slope increases, notably with an increase in the transport capacity of the runoff despite good soil resistance to erosion (erodibility index K = 0.12-0.02) and a high stability of the aggregates to water (1.6 to 2.7 mm). On the mulched treatments, runoff and erosion were negligible, even on a steep slope (LR<31mm and E<0.2 t/ha/yr). The very high proportion of covered ground (residues + plant cover + stones) on these plots (80 to 100% of plot area) proved to be very effective for infiltration and soil protection: there was no runoff with simulated cyclonic rainfall (100 mm/h for 180 minutes). On the other hand, runoff was common in the pineapple plots and soil loss was more important in the first year than in the second due to the increase in plant cover (LR=152 to 361 mm and E=29-5t/ha/yr). Simulated rainfall demonstrated the effect of ridging, which channelled the water, possibly explaining why this plot was particularly susceptible to runoff and erosion. The established banana plantations gave comparable results (Kram=2 to 4% and E=0.4 to 0.6t/ha/yr). The low level of erosion observed is the result of regular mulching with crop residues placed in windrows (S.C.=40 to 80% of plot area), an effective method of erosion control tested in African banana plantations. After the statistical analysis of the monthly results from the ten plots, two groups of factors (the R index, S.C., Dapp) and (LR and S.C.) appeared to explain the amounts of runoff and erosion respectively. It is therefore a case of Hortonian flow, involving erosion of aggregates with little dispersion.

The estimation of chemical losses on these plots shows that the more sensitive are the treatments to erosion, the greater is the loss of nutrients, which are mainly contained in the eroded sediment. Under a banana plantation, deep drainage is very active in the wet season, 32 to 37% of the seasonal rainfall. The drainage, calculated and observed by lysimetry are in fair agreement: the estimation of chemical losses by conical lysimeters seems to be correct. Moreover, this estimate indicates that in established plantation the majority of nutrients is lost by leaching and that the proportions lost are similar to those observed on an irrigated banana plantation in Côte d'Ivoire (99% of losses of Ca or of total N by drainage). ■

Growth, development and quality of fruit of the banana plant (*Musa* spp. group AAA cv 'Grande naine'). Modelling the distribution of assimilates between the fruits of a bunch

PhD thesis submitted in November 2000 to the *Institut national agronomique Paris-Grignon*, France

Alexandra Jullien

The production of bananas for export is subject to constraints of size and quality of the fruit. To be exportable from the West Indies to mainland France a fruit must have minimum values for diameter (30 mm), length (17 cm) and maturity at harvest (estimated as the duration of green life, which should exceed 20 days). Consequently, the large variability in weight and quality of fruit observed in the field results in considerable yield losses for the growers. This variability in weight depends on growing conditions but also on the position of the fruit on the bunch: the fruit on the distal part of the bunch (lower hands) are 30-40% smaller than those on the basal part (upper hands). The simultaneous study of the determination of the weight and the quality (maturity) of the fruit at harvest, described here, is therefore necessary to optimise the yield and quality of the crop.

Firstly, we determined the causes of the variation in weight of the fruit within the bunch (inflorescence). For this purpose a histological study of development was carried out to determine the chronology of the phases of cell division and of cell filling in the fruit and in the bunch. In the fruit, the phase of rapid accumulation of dry matter (filling) occurs after the end of cell division. Within the bunch, there is a lag in development between the basal hands (i.e. the upper hands which are initiated first) and the distal

hands (the lower hands which are initiated last). This lag has the consequence that the basal hands have a larger number of cells. From histological data, a conceptual scheme of development of the inflorescence was proposed.

Secondly, the source/sink ratio was modified by shading leaves (reducing the source) or by adapting cultural techniques currently used such as bagging bunches to increase the activity of the sink and removing hands to reduce the size of the sink. It appeared that the number of cells in fruits is a determining factor of the variability of weight within the bunch. On the other hand, the rate of filling of the cells is identical for all the fruit of a bunch and is determined by the source/sink ratio. A Michaelis-Menten relationship was used to relate the mean filling rate of the cells of a bunch to the mean source/sink ratio during the filling period.

Thirdly, this knowledge was introduced into a model of the distribution of assimilates within the bunch that simulates the weight of the fruit and the bunches at harvest. A relation capable of calculating the green life of fruits as a function of their age expressed as a temperature sum was added to the model, giving it a predictive value for the quality at harvest. The model thus created has a two-fold value - cognitive and predictive. First it allows us to understand and simulate plant function, using new histological knowledge and validated rules for the distribution of assimilates. It also constitutes a tool useful for diagnosing yield and as a decision aid for choosing between various cultural treatments such as bagging of bunches and removal of hands, or the determination of optimal harvest dates. ■

Physical properties of the volcanic soils of Guadeloupe used for banana growing: influence of cultural practices and their effects on rooting

PhD thesis submitted to the Faculty of Biological, Agronomic and Environmental Sciences, *Katholieke Universiteit Leuven,* Leuven, Belgium

Marc Dorel

Most of the banana plantations in Guadeloupe are situated on soils derived from recent pyroclastic rocks forming terraces on an andosol/nitisol toposequence. The practice of intensive banana growing using heavy machinery seems to be the cause of serious degradation in the structure of these soils, even though their physical properties are considered favourable for the crop. The consequences of this degradation on rooting and growth of the bananas are, however, not clearly established. An evaluation of the effect of cultural practices on the physical properties of the different soils of the toposequence is needed to propose new cultural practices which will guarantee the maintenance of soil fertility.

Firstly we have found a relationship between the porosity and water retention of the soils and their allophane and organic matter content. The effect of mechanisation of the crop on the form of the cultivated soil profile, the porosity and the hydraulic conductivity of the soil was then studied on experimental plots. Mechanisation led to the formation within the profile of contrasting structural conditions: either compact structures with low macroporosity and reduced hydraulic conductivity, or fragmented structures with high conductivity.

From the results of a survey carried out on a sample of fields under production we have tried to establish a classification of the state of the cultivated soil profile and of the rooting of the bananas. The practice of unmechanised perennial culture leads to profiles without clear structural discontinuities which allow lateral extension of the root system. The mechanisation of operations taking place during growth leads to the formation of compacted zones between the crop rows which limit the lateral extension of the root system.

We also studied the effect of the soil structure on the rooting of banana under controlled conditions. Compaction of the soil greatly reduces root biomass, rooting density and the length of the primary roots. It also causes modifications to the form and size of the cells of the root cortex.

A field experiment aimed at comparing two depths of soil cultivation showed the effect of soil tillage on root distribution and on the exploitation of soil water reserves. A crop simulation model which evaluates the effect of the soil's physical state on the functioning of the banana crop was parametrized from all the results previously mentioned.

The choice of criteria to be taken into account when planning soil preparation is then discussed. A scheme for planning soil preparation which guarantees optimal functioning of the root system and long-term conservation of soil fertility which takes into account the variability in the soil and climatic conditions of Guadeloupan banana plantations is proposed. ■

Banana streak virus

A description of the banana streak virus (BSV), prepared for Description of Plant Viruses Online, maintained by the Association of Applied Biologists, is now available on the PRO*MUSA* website under Virology working group.

Written by Andrew D.W. Geering and John E. Thomas of the Queensland Department of Primary Industries, the wide-ranging text covers topics such as transmission, serology, virus purification, particle and genome properties, relations with cells and tissues, and ecology and control. It also offers a comprehensive review of the literature on BSV, up to 2001, and contains some photographs of the symptoms.

Other descriptions of viruses are available on the websites of Description of Plant Viruses Online, http://www.dpvweb.net/, and Plant Viruses Online http://image.fs.uidaho.edu/vide/.

New method to remove latex during banana washing

The natural resin, called latex, which is exuded when the bunch is dehanded adversely affects production and fruit quality as follows:

1. Latex is insoluble in water and adheres to the fruit surface causing spots.
2. Latex supports the growth of bacteria and fungi that cause banana crown rot, one of the diseases associated with an incomplete removal of latex.

Currently, large volumes of water are needed to ensure removal of the latex from the skin and crown of the fruit.

The results of an extensive study demonstrate that the use of solvent and coagulant makes it possible to remove the latex from water. When mixed with an organic solvent, the latex is broken down and particles of polymer are released; the coagulant then attracts the material in suspension causing the latex to flocculate and settle on the bottom of the laminar flow tank. The solution comprising a mixture of natural biodegradable reagents dissolves and coagulates the latex that exudes from the hands' cut surface, which is then removed from the water. This removes the medium that supports the growth of fungi and bacteria that cause crown rot and spotting when latex adheres to the skin of the fruit.

The use of coagulant reduces considerably the volume of water needed to wash the fruit making it possible to use drinking water. Using water of better quality improves fruit quality, reduces the risk of post harvest disease, improves hygiene in the packing plant and results in substantial savings in the banana packing process. The coagulant can be sprayed or introduced drop by drop at ambient temperature, a period of 5 minutes being sufficient to remove the latex. The hands are then placed on conveyor belts and the cuts are sealed by applying a coagulating and healing solution.

Further information can be obtained from Ariatne Avila Valdes, email ariathneavila@hotmail.com

Distribution of blood disease in Indonesia

In Indonesia, blood disease caused by the bacteria *Pseudomonas celebensis* is the number one disease attacking bananas. The disease first appeared in the Selayar islands (South Sulawesi) in 1907. The bacteria has since spread to almost all the banana producing areas in Indonesia (Figure 1). Humans seem to be responsible in large part for dispersal between regions. The disease spread to Jakarta and West Java before other provinces on the island of Java, probably because of the movement of fruits to the capital. A similar pattern was observed in Maluku province. Soon after Seram island (the closest island to the provincial capital) was infected, Ambon island (where the capital is situated) was infected, but not nearby Buru island. In Solok (West Sumatra), the disease spread from the Sungai Pagu district (an infected area), from where merchants took the fruits, to the Gunung Talang district (formerly disease-free), where the fruits were graded and sorted before being transported to Jakarta, skipping the Alahan Panjang district. Because most of Indonesia's bananas are produced in backyard systems, quarantine regulations are difficult to enforce. Moreover, the normal appearance of infected plants and fruits increases the likelihood of introducing the disease to non-endemic area.

For more information, please contact Catur Hermanto and Tutik Setyawati at the Research Institute for Fruit. Jl. Raya Solok - Aripan Km 08, Solok 27301. West Sumatera – Indonesia. E-mail: rif@padang.wasantara.net.id

Two new banana species from Borneo

Two new banana species from Sarawak were found by Markku Häkkinen, a research fellow from the University of Helsinki in Finland, following a collecting mission in Borneo a few months ago.

The first species was discovered in the Bau limestone area, with field-assistant Stephen James Jossel of the Sarawak Biodiversity Centre, while the second is abundant in the Lawas area. Both species belong to the section *Callimusa*.

Häkkinen, a sea captain before turning his attention to bananas, has made seven expeditions to the Southeast region. He travelled all over Sarawak, Sabah and Kalimantan to study the wild banana species of Borneo on which he intends to write a monograph.

More studies are needed before deciding on a name for the new species and to find out whether they are resistant to the pests and diseases affecting bananas.

Source: The Borneo Post, 16 November 2002.

Improved hybrids released for commercial use in Sri Lanka and Bangladesh

Following Phase II of the International *Musa* Testing Programme (IMTP II), the Sri Lanka Department of Agriculture has identified two promising banana varieties from the germplasm collection which was transferred to the Sri Lankan Government in 1999.

Thanks to the efforts of Dr Sujatha Weerasinghe of the Horticultural Research and Development Institute, SH-3640 and FHIA-03 were recommended to the Variety Release Committee after a series of field evaluations in various locations. These accessions were given the local names of 'Kandula' and 'Pulathesi', respectively.

FHIA-03 was also released for cultivation in Bangladesh after a series of field evaluations. FHIA-03 was found to be tolerant to Fusarium wilt and to have a higher yield potential than local plantain cultivars.

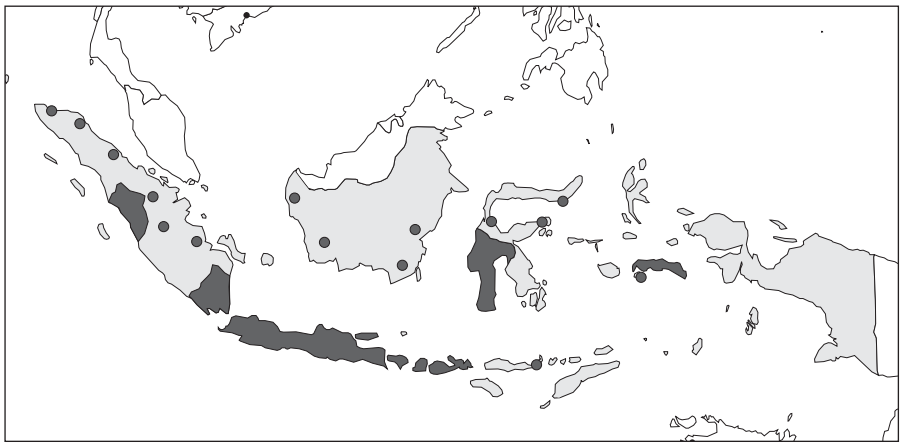

Figure 1. *Distribution of blood disease in Indonesia.*

Micropropagation of 'Nendran' and 'Poovan' varieties through inflorescence tip culture

'Nendran' and 'Poovan' are AAB cultivars that produce a low number of suckers, thus limiting the availability of suckers from elite clones and necessitating the use of *in vitro* techniques for micropropagation. Since shoot tip culture gives poor results, researchers from St. Thomas College in Pala, India, have developed a modified MS medium for the inflorescence tip culture of these varieties. In both cases, the shoots were elongated in medium containing 3 mg/L IBA and 1.5 mg/L BAP and rooted in medium with 3 mg/L IBA and 0.5 mg/L BAP (Figure 2). The shoots produced were morphologically identical to those originating from vegetative apices.

Further information is available from L. Sebastian and M.M. Mathew from the Department of Botany, St. Thomas College, Pala, Arunapuram P.O. – 686574, Kerala, India.

Banana diversity in the Middle East

The Middle Eastern region is somewhat peripheral to the banana cultivation zone in the Old World. Nevertheless, some banana cultivars have apparently been grown in this region for many centuries and questions are frequently raised regarding the extent of genetic diversity present in this region and how bananas are cultivated under such environmental conditions. In order to address these questions, INIBAP, with the financial support of FAO, organized a banana survey of Egypt, Oman and Jordan, which took place in 2002.

The survey confirmed the existence of two types of banana farmers in the region. Those who can afford the necessary investments to ensure high productivity, and those smaller scale farmers who are not faring as well and cannot rely on bananas for a regular income. The major constraints to banana production vary from country to country: water shortage in Jordan, high levels of salt in water in Oman and high labour costs in Egypt, where the presence of the banana bunchy top virus calls for a higher level of management.

This report provides an overview of banana production in each country visited, with a particular focus on the genetic diversity to be found in each country. A number of recommendations are made for each country relating to the conservation of local diversity. It was noted that the germplasm collection of the Agricultural Research Station at Salalah in Oman contains accessions that are representative of banana varieties grown in the Indian Ocean region. This is the only collection of its type, and in order to ensure the long-term conservation of this material, it is recommended that efforts be made to duplicate the accessions with INIBAP.

Figure 2. *Clusters of shoots elongated in MS_1 medium with 3 mg/L IBA and 0.5 mg/L BAP after 45 days of subculture.*

The report also provides information on the historical background of bananas in the Middle East region and includes a useful key to the identification of ABB cultivars.

The report is available on request at INIBAP Headquarters. You may also download it from http://www.inibap.org/publications/Middle-east.pdf

Tanzania *Musa* expedition

In July 2001, an expedition was organized to explore *Musa* diversity in the highland areas of Tanzania. The exploration team consisted of Dr E. De Langhe, Dr D. Karamura and Dr A. Mbwana. The team limited their prospecting to the slopes of Kilimanjaro, the Usambara hills and the South Pare hills. This zone occupies a central position for banana production in East Africa, with all the other highland areas where bananas are grown, e.g. Taita hills and Gikuyu area of Kenya, the Great Lakes region, Mbeya and Morogoro in Tanzania, being located on the periphery of this zone. It was considered that the likelihood of finding new germplasm was greatest in this area.

The mission was motivated by two major factors:

- the lack of knowledge about banana cultivars in the East African Highlands, other than the Great Lakes region
- and the growing risk that unexplored, yet potentially important material for genetic improvement would be lost due to changing farm management practices.

The expedition proved to be most successful, with 21 interesting cultivars being collected. Of these, it is likely that two may already exist as duplicates in germplasm collections. Of these 21 accessions, 10 are most probably newly acquired diploids of great potential interest for the genetic improvement of East African Highland bananas. In addition, an entirely new group of AAA-triploid bananas was identified, called the 'Ilalyi' group by the collecting team. No wild, seedy diploids were found, and the chances that such diploids exist on the continent is now considered remote. All collected accessions were planted in a field collection at the Tengeru Horticultural research Station at Arusha, Tanzania where full characterization data will be collected.

This report provides details of all accessions collected during the mission. In addition, the report provides some clarification of the hitherto rather obscure nomenclature /synonymy of cultivars in this zone, and a broad picture of how African Highland bananas in general may have been generated, is proposed.

The report is available in printed form at INIBAP Headquarters or downloadable from http://www.inibap.org/publications/Tanzania.pdf

INIBAP News

Third International symposium on Molecular and cellular biology of bananas

The Third International Symposium on Molecular and cellular biology of bananas was held on September 9-11 at KULeuven, in Belgium. The symposium was attended by 127 delegates from 33 countries. In total, 76 papers were presented during the conference, 43 as oral presentations and 33 as posters. The fields covered include genomics, gene expression and transformation, molecular pathology and disease/pest resistance, characterization and conservation of biodiversity, and biochemistry and physiology.

The invited lecturer, Dr Takuji Sasaki of the National Institute of Agrobiological Sciences and leader of the Rice Genome Research Program, gave a presentation on the sequencing of the rice genome, and a workshop on intellectual property and genetically modified organisms, chaired by Victoria Henson-Apollonio, manager of the CGIAR Central Advisory Service (CAS) on Intellectual Property, was held.

The abstracts of most of the papers presented at the symposium are posted on the PRO*MUSA* website. A few copies are available from INIBAP for those who have difficulties accessing the Web. The minutes of the PRO*MUSA* meetings held during the symposium are published in PRO*MUSA*.

CGIAR Annual General Meeting held in the Philippines

This year, the CGIAR held its Annual General Meeting (AGM) in the Philippines instead of the World Bank headquarters in Washington DC. The AGM, which started on 28 October and ended on 1 November, was

attended by representatives of CGIAR centres and donor members. It also included stakeholder and business meetings, science awards and the Crawford lecture on an agricultural topic of global significance. INIBAP's Asia-Pacific Regional Office and IPGRI-APO had one of the exhibits at the Makiling Botanic Gardens in Los Baños. Approximately 350 people visited the exhibit which featured IPGRI, COGENT, INIBAP and their collaborations in the Philippines.

At the joint press conference of the Philippines' Secretary of the Department of Agriculture, the Hon. Sec. Leonardo Montemayor, and CGIAR's Chair, Dr Ian Johnson, IPGRI's Director General, Dr Geoffrey Hawtin, made a ceremonial hand over of IMTP improved germplasm to Sec. Montemayor, followed by the signing of a material transfer agreement.

The banana varieties provided to the Philippine Government represent an important step in the continuing fight against pests and diseases which are ravaging the country's banana industry. They are part of BAPNET's National Repository, Multiplication and Dissemination Programme for the promotion and ready availability of improved varieties.

Tissue culture and virus indexing training in Taiwan

The Food and Fertilizer Technology Center, the Taiwan Banana Research Institute and the National Taiwan University, in collaboration with INIBAP's Asia-Pacific Regional Office, sponsored a training programme on tissue culture and maintenance of foundation stocks and virus indexing held on 9-20 December in Taipei.

The training was an opportunity to improve the capability to multiply and produce disease-free planting materials for those involved in the maintenance, multiplication and distribution of improved *Musa* varieties in BAPNET member countries participating in the national repository and distribution programme.

INIBAP and Lapanday: a collaboration to improve the Philippine banana industry

INIBAP's Asia-Pacific Regional Office has found a partner in the Lapanday Agricultural and Development Corporation (LADECO or simply Lapanday), a private corporation specializing in food exportations based in Davao, in the Philippines.

The partnership started when INIBAP's Asia-Pacific Regional Coordinator, Dr Agustin Molina was looking for private sector partners to do banana research for Phase III of the International *Musa* Testing Programme (IMTP III). Lapanday has an efficient tissue culture laboratory and is now supplying the national program with tissue culture of improved varieties at cost. Not only that, but Lapanday has also provided training for new partners in the field of tissue culture, nursery and field operations and maintenance. Lapanday has also supported leaf spot studies conducted by the Philippine NARS.

Global Conference on banana and plantain in India

The Association for the Improvement in Production and Utilization of Banana (AIPUB) organized, in collaboration with INIBAP and FAO, the Global Conference on Banana and Plantain held in Bangalore on 28-31 October.

The conference was attended by some 500 delegates comprising research and development experts, farmers and industry representatives from 17 countries. The theme of the conference was "Banana Production for Nutrition and Livelihood Security." Conference deliberations focused on genetic resource management and crop improvement, biotechnological advances, strategies in production technology, organic production of banana, integrated disease and pest management, postharvest management, product diversification and value addition, policy support and programmes, national and international trade and international cooperation. Two INIBAP regional coordinators presented papers: Dr Agustin B. Molina talked about R&D needs in the Asia-Pacific region and the importance in Asia of INIBAP's International *Musa* Testing Programme (IMTP) while Dr Franklin Rosales did a presentation on banana and plantain in Latin America and Caribbean countries. Dr Dirk De Waele of KULeuven presented a paper on "Nematode problems in bananas and approaches for resistance breeding."

Workshops on biotechnology applications in banana, organic banana production and banana value-added products were held concurrently with the conferences. An exhibition showcased the technological advances in the vibrant banana industry of India and the world.

Kadali Awards

During the conference, *Kadali* awards were given to Dr Emile Frison, Director of INIBAP in Montpellier, France, and Dr S. Uma, a senior scientist at the National Research Centre for Banana at Tiruchirapalli, India. This award is conferred once every two years by AIPUB in recognition of outstanding contributions to the promotion of banana research, development and utilization.

Dr Frison has provided dynamic leadership to banana research and development, improving the livelihood of millions in the Asia-Pacific region. He has been instrumental in initiating research networks and launching the Global Programme for *Musa* Improvement (PRO*MUSA*) and the Global Consortium in *Musa* Genomics.

Dr Uma has participated in the built up of the largest collection of wild, exotic and cultivated varieties of banana in Asia. She identified four new species, including the first natural tetraploid of Indian origin (Bhat Manohar) and an array of wild *balbisiana* (BB) genomes unique to the Indian subcontinent. She also contributed significantly to the taxonomy of Indian bananas.

Dr H.P. Singh, the founding Director of the National Research Centre for Banana (NRCB), and presently the Indian Government's Horticulture Commissioner, and Dr S. Sathiamoorthy, Director of NRCB, were awarded fellowships in recognition of their lifetime contributions to banana research and development.

IPGRI booth during the Philippines Day exhibit in Los Baños, Philippines on 28 October 2002. From left to right: C. Hoogendoorn (IPGRI), A. Maghuyop (INIBAP Asia-Pacific), E. Frison (INIBAP), G. Hawtin (IPGRI) and P. Sajise (IPGRI-APO).

Dr Chadha, President of AIPUB with V. Roa (INIBAP Asia Pacific) and banana growers at the INIBAP exhibit during the Global Conference on banana and plantain in Bangalore.

First BAPNET Steering Committee meeting

The first Steering Committee meeting of the Banana Asia and Pacific Network (BAPNET), formerly ASPNET, was held on 7-10 October in Los Baños, the Philippines. The conference was hosted by Dr Patricio S. Faylon, Executive Director of the Philippine Council for Agriculture, Forestry and Natural Resources Research and Development (PCARRD), and Dr Eliseo Ponce, Director of the Bureau of Agricultural Research/ Department of Agriculture (DA-BAR). The meeting was attended by 15 representatives of the member countries and institutions. This year's meeting was highlighted by the participation of two new members, Cambodia and Papua New Guinea. Among the resource persons present were Ms. Suzanne Sharrock, INIBAP Germplasm Conservation Scientist, and Dr Luigi Guarino, a DIVA software expert. Dr Jaine Reyes acted as facilitator during the workshop sessions.

Country representatives presented their industry and banana R&D status report and a special lecture on the use of the geographic information system DIVA in germplasm management was presented.

These presentations were followed by a workshop, whose objectives were to identify areas of collaboration based on NARS members strengths, constraints and opportunities, and to prioritize activities in the Asia-Pacific region for the next 3 to 5 years. The priorities identified were (1) sustainable crop management systems, (2) information exchange and management, (3) genetic diversity management, development and utilization, (4) human resource development, (5) supply chain management system, and (6) extension.

Members reiterated their interest in participating in the various INIBAP programmes/activities such as the IMTP, MGIS, and information development and exchange. The meeting emphasized the importance of INIBAP programmes that enhance BAPNET priorities. For example, the National Repository, Multiplication and Dissemination Programme, in which improved varieties are transferred to NARS for easy access by researchers and farmers, fits well with the desire of BAPNET member countries to use new varieties that are productive and resistant to the major diseases in the region.

With INIBAP's Asia-Pacific Regional Office acting as the secretariat of the network, BAPNET will develop activities to make concrete the priorities identified during the meeting. As a NARS-based network, BAPNET will work more effectively to address regional needs, enhance regional collaboration, and take full advantage of its link with INIBAP programmes.

S. Uma (NRCB) received a Kadali *award.*

2002 Awardees

During the meeting, awards were presented to individuals and institutions that have contributed significantly to banana production technology and the promotion of banana R&D programmes.

The *Pisang Raja* Award was presented to Ms Suzanne Sharrock, Germplasm Conservation Scientist at INIBAP. Her contributions date back to 1988 when she led four collecting missions in Papua New Guinea bringing back more than 200 accessions. Her generosity in sharing her expertise on *Musa* conservation and taxonomy was noted, as well as her efforts in trying to raise awareness about the importance of conserving *Musa* diversity.

Institutional Awards were given to the Lapanday Agricultural and Development Corporation (LADECO), the Department of Agriculture-Bureau of Agricultural Research (DA-BAR), Philippines, and the Philippine Council of Agriculture, Forestry and Natural Resources Research and Development (PCARRD) in recognition of their support to regional banana R&D activities.

Lastly, a special plaque was presented to Dr Ramon V. Valmayor in recognition of his outstanding contribution to banana research and his dedication to the Asia-Pacific region. In 1991, he became the first INIBAP Regional Coordinator of the Asia-Pacific region, a post he held for seven years. His research on the taxonomy and classification of *Musa* has resulted in the publication of 'Banana Names and Synonyms in Southeast Asia' and 'The Wild and Cultivated Bananas of the Philippines'. He conducted collecting missions in Vietnam, South China, Indonesia and Northeast India, which resulted in the discovery of many previously undescribed banana species and cultivars.

Third *MUSA*LAC meeting

The 3rd meeting of the Plantain and Banana Research and Development Network for Latin America and the Caribbean (*MUSA*LAC) was held in Santo Domingo, Dominican Republic from 7-10 August. CEDAF (Forest and Agriculture Development Center, Inc.) and IDIAF (Dominican Institute

Participants to the BAPNET Steering Committee meeting.

of Forest and Agriculture Research) helped with the organization of the meeting.

Thirty scientists from 13 countries (Bolivia, Brazil, Colombia, Costa Rica, Cuba, Dominican Republic, Ecuador, Honduras, Mexico, Panama, Peru, Puerto Rico and Venezuela) participated in the meeting. Up to now, Nicaragua had attended *MUSA*LAC meetings as a special guest but this year Maritza Vargas, representing Nicaragua, made an official request to *MUSA*LAC's Board which was unanimously granted, and Nicaragua became the newest member of *MUSA*LAC.

Drs Altagracia Rivera de Castillo, *MUSA*LAC's President, Rafael Ortiz, State Vice Minister of Agriculture in the Dominican government, and Franklin Rosales, Coordinator of INIBAP-LACNET, opened the meeting. The theme of this year's gathering was "Socioeconomic importance of plantain in Latin America and the Caribbean". Each representative delivered an oral and written presentation on his/her country's current situation on this subject. Fernando Sáenz from CIMPE, Costa Rica, gave a conference on "Socioeconomic importance of plantain in Latin America and the Caribbean: a regional need for information", followed by one presentation by Dr Lucía Durán, from Tolima University in Colombia and another by Dr Luis Pocasangre from INIBAP's Latin America and Caribbean Regional Office.

On the last day, *MUSA*LAC country representatives met to elect a new President and two Vice-Presidents. The elected President is Dr Jorge Sandoval from CORBANA, Costa Rica, and the two Vice Presidents are Drs Tito Díaz from CORPOICA, Colombia, and David Berroa from IDIAP, Panama. During the meeting, Guillermo Ortega Rosini, Subsecretary in the Ecuadorian government and founder member of *MUSA*LAC, was made an Honorary Life Fellow. He will soon be leaving his government post and his position as Ecuador's representative. From now on, *MUSA*LAC's Board decided to conduct an international course on banana and plantain immediately after the annual meeting, to take advantage of the presence of some *MUSA*LAC members who are also among the course lecturers.

Next year's meeting will be held in August in Ecuador under the auspices of FUNDAGRO and the Ministry of Agriculture.

Training course on clean technologies

A course on "Plantain production using clean technologies" was held in Santo Domingo, Dominican Republic, on 11-14 August, under the auspices of *MUSA*LAC and INIBAP. The purpose of this course was to demonstrate clean technologies for plantain production that increase profitability and that are economically and environmentally sustainable. More than 80 people from 12 Latin American and the Caribbean countries participated in the event.

INIBAP, in collaboration with CATIE, sponsored a workshop on "Resistance induction and clean technologies utilization of plant pest management" held in CATIE's office in Turrialba, Costa Rica, on 27-30 August. The main topics addressed were: resistance inductor molecules and induction types to pests, induction methods, resistance types, biochemical and molecular mechanisms involved in plants resistance, and alternative strategies to control pests of interest to agroforestry. Among the guest speakers who participated in the workshop were Dr Joseph Kuc from Kentucky University, USA, and Dr Richard Sikora from University of Bonn, Germany.

Acknowledgments

On 15 November, CATIE celebrated employees with many years of service to the institution. Dr Franklin E. Rosales, LACNET-Coordinator and Ms Lissette Vega, Administrative Assistant, received Recognition Certificates for, respectively, 5 and 20 years of work at CATIE. INIBAP's Latin America and Caribbean Region wishes to thank CATIE for hosting its regional office since its creation in 1987.

Suzanne Sharrock receiving a Pisang Raja *award.*

Recognition certificates were given by CATIE to F.E. Rosales, LACNET Coordinator and Ms L. Vega, Administrative Assistant.

Books etc.

*Musa*Doc CD-Rom

The fourth edition of the *Musa*Doc CDRom, *Musa*Doc 2002 is now available. Updated versions of the INIBAP databases, *MUSA*LIT, containing over 6700 bibliographic records and abstracts on *Musa*, and BRIS, the database on banana researchers (almost 900 records), are both searchable on the CDRom. All recent publications are also included in *Musa*Doc 2002 - new factsheets on banana taxonomy and diversity, among others, the report of a meeting: "Strategy for the Global *Musa* Genomics Consortium" and INIBAP annual report 2001. The CD-Rom also provides an updated summary of INIBAP's activities.

The CD-Rom, encased in a new colorful lightweight cover, is available on request from INIBAP Headquarters.

Global evaluation of *Musa* germplasm for resistance to Fusarium wilt, *Mycosphaerella* leaf spot diseases, and nematodes: In-depth evaluation. INIBAP Technical Guidelines 6

Jean Carlier, Dirk de Waele and Jean-Vincent Escalant in collaboration with the PRO*MUSA* Fusarium, Sigatoka and Nematology working groups

Edited by Anne Vézina and Claudine Picq

ISBN: 2-910810-52-6

These Technical Guidelines replace Technical Guidelines 1 (*Screening of* Musa *Germplasm for resistance and tolerance to nematodes)* and 3 (*Evaluation of* Musa *germplasm for resistance to Sigatoka diseases and Fusarium wilt*) as far as in-depth evaluations are concerned. Technical Guidelines for performance evaluations, which were also part of Technical guidelines 3, will appear shortly.

These changes were made to reflect the apparition of a third disease caused by a *Mycosphaerella* fungus (eumusae leaf spot disease), to simplify the guidelines, and to group under one cover the evaluations for the main pests and diseases affecting bananas.

These guidelines have been written with the collaboration of the Fusarium, Sigatoka and Nematology working groups of PRO*MUSA* and INIBAP staff with the objective of helping researchers to design their trial, choose the appropriate location for the trial, and evaluate selected genotypes for resistance and tolerance to various diseases. Field forms are included to help data collection.

INIBAP thanks all the scientists who contributed to these guidelines.

These guidelines are also available in French and Spanish.

Book review

The Wild and Cultivated Bananas of the Philippines

Ramon V. Valmayor, René Rafael C. Espino and Orlando C. Pascua. 2002. Philippine Agriculture and Resources Research Foundation, Inc. (PARRFI).

ISBN 971-92540-1-7.

Reviewed by Michael G. Price

More than two-thirds of this hard-bound volume is devoted to 87 cultivars from the Philippines. Each entry consists of a list of morphological and horticultural characteristics, and a line drawing of the whole plant with close-ups of the leaf-base, flower bud, bracts and bunch. Having all this data and imagery visible at a glance is a great convenience.

There are also chapters on previous and current banana classification schemes, nomenclature, and genetics, which focus on, but are not limited to the Philippines. The book also contains valuable indices and lists, and an appendix with descriptions of two new species and a subspecies recently described by Valmayor.

Since bananas are native to Southeast Asia, the Philippines, with over 7000 islands, is a very important part of that natural range. Furthermore, many of the superior varieties, which might possibly have been first discovered elsewhere by ancient horticulturists, are now grown in the Philippines, and are therefore included in this book.

Of the 87 cultivars treated in detail, an astonishing 41 of them had not been previously characterized. Among these newly described ones are several that seem to be especially promising, and my personal favorite, from glancing over the descriptions, is the cultivar 'Manang' with small but extremely sweet and aromatic fruit, and also an early bearer. It may have excellent commercial potential, or it might just be wonderful to have in your own backyard garden.

In addition to these 41 new cultivars, the book is also the first publication to mention several discoveries. One example is the recognition that *Musa peekelii* of Papua New Guinea and the Bismarck Archipelago is identical to the plant known as 'Pakel' in Palawan, in the Philippines, a range extension of about 2000 miles. Remarkably, this wild species has almost the same vernacular name in both places, thus indicating a prehistoric link.

Several noteworthy bananas not included among the 87 described cultivars because they are recent introductions to the country, are also discussed and shown. From Malaysia has come the fascinating 'Pisang seribu' which was once proposed as a separate species by the Dutch botanist Backer. The Malay name means thousand-fruit banana.

This fine banana book will be valuable to anyone with a serious interest in the subject, whether they are botanists, horticulturists, growers, or even dietitians and doctors. For example, in relation to the claim that plantains are very effective in lowering blood pressure, preventing and treating ulcers, and decreasing the risk of heart disease, among other things, the authors explain that plantain is an inexact botanical term and that there many are genetically different. Some plantains may turn out to have better therapeutic qualities than others. The background information for doing research on that subject is here ready and waiting.

Address for orders: PARRFI, c/o PCARRD Headquarters, Paseo de Valmayor, 4030 Los Baños, Laguna, Philippines; fax (049) 536-0016; email parrfioffice@pacific.net.ph
$50 + airmail postage (Asia $16, Pacific and Middle East $20, Europe and North America $22, Africa and Latin America $23).

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  Books: Stover R.H. & N.W. Simmonds. 1987. Bananas (3rd edition). Longman, London, United Kingdom.

  Articles (or chapters) in books: Bakry F. & J.P. Horry. 1994. *Musa* breeding at CIRAD-FLHOR. Pp. 169-175 *in* The Improvement and Testing of *Musa*: a Global Partnership (D.R. Jones, ed.). INIBAP, Montpellier, France.

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Publications from INIBAP

The following publications are available from headquarters:

INIBAP 2002. Networking bananas and plantains: Annual Report 2001.

INIBAP 2002. The Global *Musa* Genomics Consortium. Strategy for the Global *Musa* Genomics Consortium: Report of a meeting held in Arlington, USA, 17-20 July 2001.

INIBAP/CTA/CIRAD 2001. J. Daniells, C. Jenny, D. Karamura & K. Tomekpe. *Musa*logue: a catalogue of *Musa* germplasm. Diversity in the genus *Musa* (E. Arnaud & S. Sharrock, compil.).

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