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Vol. 15 No. 1 & 2
June-December 2006
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Yes, we are changing again… but not immediately! Those of our readers who appreciate the present format of InfoMusa can look forward to one more issue with the same ‘look and feel’ after this one. Meanwhile, those who have contributed scientific articles and are waiting patiently for a response can expect to hear soon from our Editor whether theirs will be among the next batch to be published. However, the urgent message to would-be contributors is: please do not send further manuscripts – at least until you hear from us again, via an email alert, an announcement on our website, or in the next editorial of InfoMusa.

So, what is happening? On the one hand, we have accumulated a considerable back-log of potential articles. As we have explained in an earlier editorial, we take very seriously our mission to raise the quality of contributed articles to achieve adequate standards of scientific reporting. However, the magnitude of this task has been steadily overwhelming the capacity of our editorial committee to review and revise manuscripts, as well as outstripping the time that our Editor can invest in working with authors to respond to the reviewers’ recommendations for improving the texts. Hence we are imposing this moratorium on new contributions. We plan to produce a larger-than-usual edition, towards the end of 2007 or at the beginning of 2008 to accommodate as many as possible of the contributed manuscripts. In the meantime, we apologise for the delay in reviewing articles and we ask for the continued patience of authors with articles ‘in the pipeline’.

On the other hand, we have also concluded that the time has come for a further evolution in both our mission and methods. Those of our readers who also watch our website will have noticed that INIBAP has now become Bioversity International and those who work at, or are closely involved with, the CGIAR Centres will know that ‘the system’ is placing ever more emphasis on developing more effective ‘impact pathways’ through which the products of agricultural research can be adopted and can contribute to development goals. As part of our own continuing search for greater impact and cost-effectiveness, we have decided to make more use of internet technology, while preserving the on-paper format for those who have limited internet access or who simply like to have a magazine to read.

While the next issue of InfoMusa is going to press, we shall start work on a web-based information platform, provisionally called InfoMus@. This will be organized by themes, including those identified in earlier readers’ surveys, and will address our main aim of keeping readers in the research-and-development community abreast of developments in the world of bananas. It will include news, opinions and thematic fora addressing specific issues of interest to the development community. At intervals, probably once a year, we shall prepare a digest of the best material contributed to the information platform and publish it in magazine format – the new InfoMusa.

Regarding the scientific articles, we would be interested in receiving feedback from readers, but our impression is that there are now a multitude of international, regional and national refereed journals where articles on banana research could better be published. We feel that we can best invest our own limited resources in guiding our partners to such publications, through bibliographic reviews and selective literature alerts. We are also planning to organize at least one banana research meeting every year, under the auspices of ProMusa and the Banana and Plantain Section of the International Society for Horticultural Sciences. The proceedings will be published in the Society’s journal. So there are plenty of opportunities to publish interesting results!

In the proposed new way of working, we shall be using the email a lot more, namely to let you know of new material being posted on the InfoMus@ information platform. It is therefore particularly important that we have your current email address on file. If you have not heard from us recently (for instance about this year’s banana conference in South Africa) then it probably means that our records are out of date and you need to notify us by sending a message to bioversity-france@cgiar.org. This would also be a good moment to check your entry in BRIS, our database of banana researchers, and update your records there too.

We look forward to continuing to work with you - and to serve the information needs of the banana research and development community - through these diverse media.

Richard Markham, director of Commodities for Livelihoods
Effect of associated plant species on banana nematodes

D. De Waele, R. Stoffelen and J. Kestemont

Several plant species are found growing with bananas. The weed Syngonium podophyllum (arrowhead vine), which climbs up the pseudostem of a banana plant, can cause serious problems in plantations. The cover crops Geophila repens and Arachis pintoi are grown with bananas to reduce erosion and increase soil fertility (Stover and Simmonds 1987, Humphreys and Partridge 1995). Sorghum bicolor (forage sorghum) and Sorghum vulgare var. sudanense are used in rotation with bananas to increase soil fertility (Temisien 1989, Temisien and Ganry 1990). Tagetes spp. (marigolds) have long been known to possess nematicidal activity (Reynolds et al. 2000, Ploeg 2002).

Little is known about the positive or negative effects these plants might have on the populations of banana nematodes. The species A. pintoi is reported to reduce the galling of Meloidogyne incognita and Meloidogyne arabica on tomato (Domínguez-Valenzuela et al. 1990, Marban-Mendoza et al. 1992) and to decrease Rotylenchulus reniformis numbers on coffee (Herrera and Marban-Mendoza 1999).

Sorghum is a common name for different Sorghum species and cultivars. Consequently, contradictory information is found. Sorghum is reported as a host for Radopholus similis (Keetch 1972, Inomoto 1994), but is used as rotation crop to reduce the number of R. similis in banana fields (Temisien and Melin 1989). Sorghum is also reported as a useful rotation crop to reduce levels of R. reniformis based on its non-host character (Dunn 1990). However, Dao (1972) observed maintenance of a R. reniformis population on Sorghum. Sorghum vulgare is reported as host for Helicotylenchus dihystera (Rao and Swanup 1974), but gradations in susceptibility are observed for S. bicolor (Jain and Hasan 1987). Sorghum is used as rotation crop for Meloidogyne spp. (Dunn 1990, McSorley and Gallaher 1992), but M. incognita can reproduce very well on S. bicolor (Carter and Nielo 1975).


Residues from previous planting can also affect nematode numbers. Previous planting with Tagetes spp. has been reported to reduce infection by Pratylenchus zeae on maize (Jordaan and De Waele 1988) and root galling on tomato by Meloidogyne arenaria, Meloidogyne hapla, M. incognita, and Meloidogyne javanica (Ploeg 1999).

The objectives of this study were 1) to determine the host suitability to banana nematodes of six selected plant species often found growing with bananas, 2) to study the effect of plant residues on nematode levels in bananas, and 3) to investigate the effect on nematode levels of competition between the selected plant species and bananas.

Materials and methods

Tissue-culture plants of the cultivar “Ecuador dwarf” (AAA, Cavendish group), cuttings of G. repens, A. pintoi, S. podophyllum, and seeds of S. bicolor, S. vulgare and Tagetes erecta were used as source of nematode-free planting material. This plant material was transferred to plastic bags, 20 cm in diameter, filled with field soil (28% sand, 44% silt, 28% clay) infested with the banana nematodes R. similis, H. multicinctus, Meloidogyne spp. and R. reniformis. The bags were maintained in a shadehouse and irrigated daily. For the host suitability test, seedlings and cuttings were thinned to two plants of G. repens and S. podophyllum, three plants of A. pintoi, five plants of S. bicolor and S. vulgare and seven plants of T. erecta.

For the plant residue test, banana plants were planted in the same soil as in the host suitability test. For the competition test, a banana plant was grown together with a plant of G. repens, A. pintoi, S. podophyllum, S. bicolor, S. vulgare or T. erecta in bags

Nematodes
filled with infested soil from the field. Eight replications per plant species or combination of plant species were analysed in each experiment.

Plants were harvested four weeks after planting. The number of nematodes per root system and per gram of fresh roots were determined for each plant. The entire root system was weighed and cut into 2 cm pieces. The roots were macerated in a blender for 20 seconds or 10 seconds if the root weight was less than 10 g. The nematodes were concentrated using 150, 75 and 30 µm pore sieves. The nematode suspension was purified by sugar centrifugation (Hooper 1990) and the nematodes were collected using a 30 µm pore sieve.

To extract the nematodes from the soil, water was added to 100 g of soil. The nematodes were then passed through 150 and 30 µm pore sieves. Material retained on the 150 µm pore sieve was discarded and the nematodes retained by the 30 µm pore sieve were collected. The nematode suspension was purified using the centrifugation-sieving method (Hooper 1990).

Prior to statistical analysis nematode numbers were log_{10} (x+1) transformed. Data that were not normally distributed due to the high number of zero values were analysed with a nonparametric test, the Kruskal Wallis test, the Method of Multiple Comparisons (Siegel and Castellan 1988) and the Method of Multiple Comparisons (Siegel and Castellan 1988) was used to compare them. Data that were not normally distributed and had homogeneous variances were subjected to an analysis of variance (ANOVA). The means were separated by Tukey’s test at p≤0.05 (Spjotvoll and Stoline 1973).

Table 1. Host suitability to banana nematodes of various plants species and the banana cultivar Ecuador dwarf four weeks after planting in nematode-infested soil.

<table>
<thead>
<tr>
<th>Plant or Cultivar</th>
<th>Root fresh weight (g)</th>
<th>Number of nematodes per root system</th>
<th>Infected plants (%)</th>
<th>Number of nematodes per g of roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geophila repens</td>
<td>3.9</td>
<td>35 ab</td>
<td>63</td>
<td>9 a</td>
</tr>
<tr>
<td>Arachis pintoi</td>
<td>1.9</td>
<td>40 ab</td>
<td>71</td>
<td>8 a</td>
</tr>
<tr>
<td>Syngonium podophyllum</td>
<td>5.3</td>
<td>33 ab</td>
<td>10</td>
<td>6 a</td>
</tr>
<tr>
<td>Sorghum bicolor</td>
<td>17.6</td>
<td>110 abc</td>
<td>65</td>
<td>10 ab</td>
</tr>
<tr>
<td>Sorghum vulgare</td>
<td>11.1</td>
<td>111 bc</td>
<td>35</td>
<td>25 a</td>
</tr>
<tr>
<td>Tagetes erecta</td>
<td>3.1</td>
<td>6 a</td>
<td>20</td>
<td>1 a</td>
</tr>
<tr>
<td>Ecuador dwarf</td>
<td>4.6</td>
<td>486 c</td>
<td>100</td>
<td>112 b</td>
</tr>
</tbody>
</table>

Data were log_{10} (x+1) transformed for analysis. Means in the same column followed by the same letter do not differ significantly at p≤0.05, according to the method of multiple comparisons.

Results

Host suitability test

Four weeks after planting, all plant species evaluated were infected with nematodes (Table 1). The highest number of nematodes was found in the roots of ‘Ecuador dwarf’. The number of nematodes in the root system was significantly lower on G. repens, A. pintoi, S. podophyllum and T. erecta than on the banana plant. Both Sorghum species were as susceptible to banana nematodes as ‘Ecuador Dwarf’. Although all plant species were infected with nematodes, the percentage of infected plants varied between 25 and 100%, with high levels in both Sorghum species and the banana cultivar. Compared to the banana plant, the number of nematodes per gram of roots were significantly lower on all the evaluated species, except S. vulgare.

The following nematode species were extracted from the roots of the species under study: R. similis, H. multicinctus, Meloidogyne spp. and R. reniformis (Table 2). Syngonium podophyllum was free of R. similis and R. reniformis, while R. similis and Meloidogyne spp. were absent in the roots of T. erecta. The numbers of H. multicinctus

Table 2. Levels of nematodes on various plant species and the banana cultivar Ecuador dwarf 4 weeks after planting in nematode-infested soil.

<table>
<thead>
<tr>
<th>Plant or Cultivar</th>
<th>Radopholus similis</th>
<th>Helicotylenchus multicinctus</th>
<th>Meloidogyne spp.</th>
<th>Rotylenchulus reniformis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nematodes g of roots</td>
<td>Infected plants (%)</td>
<td>Nematodes g of roots</td>
<td>Infected plants (%)</td>
</tr>
<tr>
<td>Geophila repens</td>
<td>1 ab</td>
<td>13</td>
<td>1 a</td>
<td>25</td>
</tr>
<tr>
<td>Arachis pintoi</td>
<td>2 ab</td>
<td>14</td>
<td>12 ab</td>
<td>43</td>
</tr>
<tr>
<td>Syngonium podophyllum</td>
<td>0 a</td>
<td>0</td>
<td>3 ab</td>
<td>57</td>
</tr>
<tr>
<td>Sorghum bicolor</td>
<td>1 ab</td>
<td>38</td>
<td>1 a</td>
<td>25</td>
</tr>
<tr>
<td>Sorghum vulgare</td>
<td>1 ab</td>
<td>50</td>
<td>3 ab</td>
<td>100</td>
</tr>
<tr>
<td>Tagetes erecta</td>
<td>0 a</td>
<td>0</td>
<td>1 a</td>
<td>13</td>
</tr>
<tr>
<td>Ecuador dwarf</td>
<td>20 b</td>
<td>88</td>
<td>48 b</td>
<td>100</td>
</tr>
</tbody>
</table>

Data were log_{10} (x+1) transformed for analysis. Means in the same column followed by the same letter do not differ significantly at p≤0.05, according to the method of multiple comparisons.
were significantly lower in the roots of G. repens, S. bicolor and T. erecta than in the banana roots. The number of Meloidogyne spp. was significantly lower in the roots of A. pintoi than in the banana roots.

Four weeks after planting, all the nematode species were still present in the soil (Table 3). The numbers of nematodes recovered from the soil were significantly lower after growing A. pintoi, S. bicolor, S. vulgare and T. erecta than after growing bananas. Rotylenchulus reniformis and H. multicinctus were more common in the soil than R. similis and Meloidogyne spp. Differences in the number of nematodes per 100 gram of soil were due to the differences in the number of R. reniformis. Although the soil surrounding some of the plants was free of R. similis and/or Meloidogyne spp., no significant differences in the number of these nematodes were observed due to the low frequency of these nematodes.

Plant residue test

The number of nematodes in the roots of the banana cultivar after cultivation of the six species was compared with the number of nematodes after successive banana cultivation (Table 4). No significant differences in the numbers of nematodes per root system and per gram of roots were observed. Radopholus similis was not found in banana roots after cultivation of A. pintoi and of S. podophyllum. However, no significant differences were found since small numbers of R. similis were recovered in the banana roots of the other treatments as well. Only two significant differences were found: a higher number of H. multicinctus was recovered from S. bicolor than from S. podophyllum and a higher number of Meloidogyne was found in the roots of G. repens than of S. vulgare.

Competition test

The numbers of nematodes in the banana roots were always higher than the number of nematodes in the other plant in the same pot (Table 5). Radopholus similis, H. multicinctus and Meloidogyne spp. were found in the roots of all plants, except G. repens and T. erecta. Significantly lower numbers of R. similis were found in banana plants grown together with T. erecta compared to banana plants grown with G. repens.

Table 3. Number of nematodes recovered from 100 gram of soil 4 weeks after planting various plant species in nematode-infested soil.

<table>
<thead>
<tr>
<th>Plant residue test</th>
<th>Total number of nematodes</th>
<th>Radopholus similis</th>
<th>Helicotylenchus multicinctus</th>
<th>Meloidogyne spp.</th>
<th>Rotylenchulus reniformis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geophila repens</td>
<td>402 ab</td>
<td>0</td>
<td>67</td>
<td>13</td>
<td>321 ab</td>
</tr>
<tr>
<td>Arachis pintoi</td>
<td>281 a</td>
<td>13</td>
<td>100</td>
<td>0</td>
<td>194 a</td>
</tr>
<tr>
<td>Syngonium podophyllum</td>
<td>461 ab</td>
<td>0</td>
<td>117</td>
<td>8</td>
<td>336 ab</td>
</tr>
<tr>
<td>Sorghum bicolor</td>
<td>428 ab</td>
<td>6</td>
<td>59</td>
<td>6</td>
<td>352 a</td>
</tr>
<tr>
<td>Sorghum vulgare</td>
<td>387 a</td>
<td>24</td>
<td>65</td>
<td>18</td>
<td>281 a</td>
</tr>
<tr>
<td>Tagetes erecta</td>
<td>270 a</td>
<td>0</td>
<td>65</td>
<td>0</td>
<td>205 a</td>
</tr>
<tr>
<td>Ecuador dwarf</td>
<td>1347 b</td>
<td>24</td>
<td>123</td>
<td>18</td>
<td>1183 b</td>
</tr>
<tr>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Data were log 10 (x+1) transformed for analysis.
NS = not significant according to the Kruskal Wallis rank test.
Means in the same column followed by the same letter do not differ significantly at p≤0.05, according to Tukey’s test.

Table 4. Effect of crop residues on nematode populations in the roots of the banana cultivar Ecuador dwarf 4 weeks after planting.

<table>
<thead>
<tr>
<th>Previous crop</th>
<th>Nematodes per g of root system</th>
<th>Nematodes per g of roots</th>
<th>Radopholus similis per g of roots</th>
<th>Helicotylenchus multicinctus per g of roots</th>
<th>Meloidogyne spp. per g of roots</th>
<th>Rotylenchulus reniformis per g of roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geophila repens</td>
<td>357</td>
<td>35</td>
<td>1</td>
<td>5 ab</td>
<td>19 b</td>
<td>12</td>
</tr>
<tr>
<td>Arachis pintoi</td>
<td>389</td>
<td>36</td>
<td>0</td>
<td>5 ab</td>
<td>17 ab</td>
<td>14</td>
</tr>
<tr>
<td>Syngonium podophyllum</td>
<td>322</td>
<td>39</td>
<td>0</td>
<td>5 a</td>
<td>19 ab</td>
<td>15</td>
</tr>
<tr>
<td>Sorghum bicolor</td>
<td>314</td>
<td>48</td>
<td>1</td>
<td>14 b</td>
<td>11 ab</td>
<td>21</td>
</tr>
<tr>
<td>Sorghum vulgare</td>
<td>255</td>
<td>30</td>
<td>1</td>
<td>11 ab</td>
<td>7 a</td>
<td>15</td>
</tr>
<tr>
<td>Tagetes erecta</td>
<td>276</td>
<td>35</td>
<td>3</td>
<td>6 ab</td>
<td>13 ab</td>
<td>15</td>
</tr>
<tr>
<td>Ecuador dwarf</td>
<td>311</td>
<td>36</td>
<td>12</td>
<td>8 ab</td>
<td>19 ab</td>
<td>10</td>
</tr>
<tr>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Data were log 10 (x+1) transformed for analysis.
NS = not significant according to the Kruskal Wallis rank test or ANOVA.
Means in the same column followed by the same letter do not differ significantly at p≤0.05, according to Tukey’s test.
Table 5. Effect of competition on nematode infection 4 weeks after planting in infested field soil.

<table>
<thead>
<tr>
<th>Species</th>
<th>Total number of nematodes per root system</th>
<th>Radopholus similis per g of roots</th>
<th>Helicotylenchus multicinctus per g of roots</th>
<th>Meloidogyne spp. per g of roots</th>
<th>Total number of nematodes in banana cultivar</th>
<th>Radopholus similis per g of roots</th>
<th>Helicotylenchus multicinctus per g of roots</th>
<th>Meloidogyne spp. per g of roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geophila repens + Ecuador dwarf</td>
<td>591</td>
<td>53 b</td>
<td>103</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arachis pintoi + Ecuador dwarf</td>
<td>594</td>
<td>40 ab</td>
<td>83</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sorgothum vulgare + Ecuador dwarf</td>
<td>439</td>
<td>17 ab</td>
<td>48</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tagetes erect + Ecuador dwarf</td>
<td>424</td>
<td>24 ab</td>
<td>59</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sorghum bicolor + Ecuador dwarf</td>
<td>673</td>
<td>32 ab</td>
<td>92</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sorghum bicolor + Ecuador dwarf</td>
<td>320</td>
<td>11 a</td>
<td>53</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data were log_{10} (x+1) transformed for analysis.

NS = not significant according to the Kruskal Wallis rank test or ANOVA.

Means in the same column followed by the same letter do not differ significantly at p≤0.05, according to Tukey’s test.

Discussion

Based on the number of nematodes per gram of root, *G. repens*, *A. pintoi*, *S. podophyllum*, *S. bicolor* and *T. erecta* grown in nematode-infested soil were less susceptible to nematodes than ‘Ecuador dwarf’. However, the number of nematodes in the root system of *S. bicolor* was not significantly different from the number of nematodes in the banana root system. Jordaan and De Waele (1988) also mentioned that the classification of host suitability of a given plant on the basis of nematodes per root system and nematodes per root unit can differ. In this study, the classification is based on nematode densities. The host status of *S. vulgare* is not clear since the number of nematodes per gram of roots was not significantly different to the one in bananas and the other five evaluated species.

The cover crop *G. repens* can be considered as a poor host for *H. multicinctus*. However, the related species *Geophila macropoda* is reported as host for *Helicotylenchus* and *R. similis* based on the presence of more than 2.1 nematodes per gram of roots (Araya 1998).

The cover crop *A. pintoi* is a poor host for *Meloidogyne* spp. and suppressed the number of *R. reniformis* in the soil. This study confirms the host status of *A. pintoi* for *R. similis* (Araya 1998).

The non-host status of *S. podophyllum* for *R. similis* (Edwards and Wehunt 1971) can be extended to *R. reniformis*.

The rotation crop *S. bicolor* can be considered as a poor host for *H. multicinctus*. Both *Sorghum* species suppressed the number of *R. reniformis* in the soil.

*Tagetes erecta* can be considered as a poor host for *H. multicinctus* and a non-host for *R. similis* and *Meloidogyne* spp. In addition, the population of *R. reniformis* in the soil was suppressed by this species. The absence of nematodes in the roots of *T. erecta*, when grown in combination with banana plants, confirms the low susceptibility of this species to banana nematodes.

No effect of plant residues on nematode levels in banana roots were observed, even though several species were poor hosts for banana nematodes. In the present study, the precultivation period of four weeks was probably too short to allow residues to have an effect on nematode numbers.

When another plant was grown in the presence of banana, most of the nematodes were recovered from the banana roots. *Geophila repens* and *T. erecta* were even free of *R. similis*, *H. multicinctus* and *Meloidogyne* spp., although these nematode species were observed in the banana roots. Preference of *R. similis* for banana roots over those of *Geophila macropoda* had already been observed by Araya (1998) when *Geophila* was grown in presence of the banana cultivar ‘Grande naine’.

Conclusion

*Geophila repens*, *A. pintoi*, *S. bicolor* and *T. erecta* show promise as cover crops, rotation crops or intercrops that would not increase the population of banana nematode. This potential should be validated in field trials.
of longer duration. The weed *S. podophyllum* cannot be considered as a reservoir for *R. similis* and *R. reniformis*. The host status of *S. vulgaris* has to be clarified before this crop can be introduced in rotation schemes.

**Acknowledgements**

The third author thanks Standard Fruit Company for the opportunity to prepare a M.Sc. thesis in the Honduran plantation in 1995. This research was financed by the Catholic University of Leuven (K.U.Leuven).

**References**


Two diseases of bananas are the subject of this review: Moko bacterial wilt and Bugtok. Moko bacterial wilt is found in Latin America and the Caribbean, where it occurs on cooking bananas of the Bluggoe subgroup (ABB) and dessert bananas belonging to the Cavendish group (AAA). Bugtok is a disease found exclusively in the Philippines, where it attacks the cooking bananas ‘Saba’ and ‘Cardaba’. It was first reported in 1965 (Roperos 1965) and later fully described (Soguilon et al. 1994 a and b, Soguilon et al. 1995).

Because of marked differences in symptoms and modes of transmission, it was at first suspected that the two diseases were caused by distinct agents. However, later work, including a variety of DNA-based diagnostic methods and comparative pathogenicity tests, has shown conclusively that the two diseases are caused by the same agent: Ralstonia solanacearum race 2. In the hierarchical classification of Fegan and Prior (2006) all strains of R. solanacearum race 2 affecting banana and plantain are classified into phylotype II and into several sequevars based upon sequence differences in conserved regions of the endoglucanase gene. Sequevars 3, 4 and 6 correspond, respectively, to the three multi locus genotypes (MLGs), MLG 24, 25 and 28 previously described by Cook et al. (1989). In the Philippines, all isolates of R. solanacearum from Moko bacterial wilt and Bugtok conform to sequevar 3 and MLG 24. They form a monomorphic group suggesting a clonal origin and a relatively recent introduction to the Philippines (Fegan 2005, Llagan et al. 2003).

Despite differences between Moko bacterial wilt and Bugtok, the use of different common names for the diseases is a source of confusion and requires justification. The objective of this review is to explain the origin of the common names and, in particular, to describe the key differences in disease transmission and control, on which there are conflicting statements in the literature.

Disease common names
The Committee on Common Names of Plant Diseases set up by the International Society for Plant Pathology has released the results of its conclusions on banana diseases: Common names of banana diseases and their causal agents (www.isppweb.org/names_banana_common.asp). The recommended common name for the wilt of dessert and cooking bananas caused by Ralstonia solanacearum race 2 in Latin America and the Caribbean, and in Mindanao, southern Philippines, is Moko bacterial wilt, derived from the name of the banana most severely affected by the disease in Trinidad in the early years of the 20th century.

The recommended common name for the fruit rot of the cooking bananas ‘Saba’ and ‘Cardaba’ in the Philippines is Bugtok, or bacterial hard pulp. Hardening of the fruit pulp is a distinctive feature of this disease. Three names have been used in the Philippines to describe this disease and all refer to hardening of the fruit pulp: Bugtok, derived from the Cebuano or Visayan dialect is used by people in Mindanao, and has priority over tapurok and tibaglon, which are commonly used in the Visayan islands and specifically in Negros Oriental (M. Natural pers. comm.)

In the western hemisphere, Moko bacterial wilt occurs on the cooking bananas of the Bluggoe subgroup (‘Bluggoe’, ‘Moko’, ‘Cachaco’ and ‘Chato’) as well as other subgroups. Transmission of the disease occurs principally through insects visiting the male flowers. Once the flower of any susceptible cultivar has been infected, the bacteria move through the vascular system of the peduncle and pseudostem to the rhizome and other organs, which can lead to mechanical transmission by machete during pruning operations. When rhizomes from diseased fields are used to establish new plantations, the disease is readily transmitted (French and Sequeira 1968). Both Moko bacterial wilt and Bugtok are
insect-transmitted diseases that differ with regards to symptoms and other modes of transmission on cooking bananas (Table 1).

The justification for Bugtok as a common name distinct from Moko bacterial wilt is based on the differences observed in fruit symptoms, the lesser extent to which it affects the other parts of the plants, absence of wilt and lack of transmission through suckers. However the relative importance of host genotype, environment and strain of the pathogen in the expression of the different disease symptoms in the Philippines and Latin America is not understood. It is not known, for example, whether the symptoms of Bugtok would be the same as in the Philippines if ‘Saba’ and ‘Cardaba’ were grown on a large scale in Latin America, or whether the symptoms induced by MLGs 25 and 28 on these cultivars would be the same or different from those induced by MLG 24 in the Philippines.

**Transmission of Moko bacterial wilt and Bugtok**

In Latin America and the Caribbean, Moko bacterial wilt is locally transmitted on dessert bananas and ‘Bluggoe’ by the use of machetes and other cutting implements, root to root transmission, movement of contaminated soil and flood water. Over longer distances, it is transmitted by insects, particularly on ‘Bluggoe’. Wardlaw (1972) states that insect transmission has occurred over distances of 90 km in Colombia and Venezuela. It is not known whether this is solely the result of stepwise incremental spread from infected to healthy inflorescences.

*R. solanacearum* does not produce desiccation-resistant cells and prolonged survival of the cells present in the bacterial ooze that adheres to the bodies of insects is unlikely. Whether the insects reported to be involved in insect transmission are capable of travelling in one step the distances reported for Moko bacterial wilt is not clear from the literature. Nothing is known about the mechanism of insect transmission or whether some other vector is involved (Buddenhagen and Elsasser 1962).

Moko bacterial wilt has been moved across national boundaries on infected planting material (Buddenhagen 1961, Hunt 1987, Lehmann-Danzinger 1987). The presence of the disease in Honduras and on the Caribbean coast of Panama followed the introduction of planting material from areas already having the disease (Buddenhagen 1961). Black and Delbeke (1991) state that Moko bacterial wilt in Belize was almost certainly introduced from neighbouring Guatemala on planting material of Bluggoe. French and Sequeira (1968) attributed to insect transmission the progression, along the tributaries of the Amazon in Peru, of the disease on ‘Bluggoe’ and similar cooking bananas. They warned against movement by boat of infected bunches because of the possibility that the exudates could be transmitted by insects, machetes or river water. Other references in the literature to the possible dispersal of the disease by fruit transport (e.g., Hunt 1987) relate to cooking bananas. Literature suggesting that Moko bacterial wilt has spread to dessert bananas in new localities through infected fruit has not been found.

There is some risk that other plants susceptible to the disease, such as *Heliconia* spp., and possibly *Dieffenbachia* spp. and tannia (Hunt 1987), could transmit Moko bacterial wilt. The disease was detected at Bombay airport in 1990 in a consignment of *Heliconia* spp. from Hawaii (Reddy and Nikale 1992) and on plants, also from Hawaii, in a post-entry quarantine nursery in Cairns, Australia (Hyde et al. 1992).

---

**Table 1. Symptoms and alternative mode of transmission of Ralstonia solanacearum race 2 in cooking bananas.**

<table>
<thead>
<tr>
<th>Hosts</th>
<th>Fruits</th>
<th>Leaves</th>
<th>Transmission through suckers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>External symptoms</td>
<td>Internal symptoms</td>
<td></td>
</tr>
<tr>
<td>Saba and Cardaba in the Philippines</td>
<td>No symptoms</td>
<td>Hardening and black or red discoloration of the pulp</td>
<td>No symptoms</td>
</tr>
<tr>
<td>Bluggoe and related cooking bananas in the Americas</td>
<td>Symptomless to fruit malformation and premature ripening (yellowing) of a few fingers</td>
<td>Brown viscous rot or grey and dry</td>
<td>Wilting of the foliage in adult plants</td>
</tr>
</tbody>
</table>

Based on descriptions by Black and Delbeke (1991), French and Sequeira (1968) and Soguilon et al. (1994 a and b, 1995)
Instances of introduction and establishment on dessert bananas of Moko bacterial wilt following introduction of the disease on an alternative host such as Heliconia have not been reported. Although the disease has existed on Heliconia in Hawaii for almost 20 years, there have been no reports of Moko bacterial wilt on dessert bananas in Hawaii. There are conflicting reports on the first introduction of Moko bacterial wilt in the Philippines. Some authors have stated that the disease had been brought in on rhizomes from Honduras around 1968 (Buddenhagen 1986, 1994, Stover 1972), but this cannot have been the first introduction because Bugtok had been reported several years earlier (Roperos 1965). Soguilon et al. (1994a) state that Bugtok was known in Mindanao since the early 1950s. According to Liagan et al. (2003), anecdotal and circumstantial evidence points to the introduction from Central America of contaminated banana planting material in the early 1940s. Bugtok disease occurs throughout the Philippines (Molina 1996), but its widespread distribution is unlikely to have been the result of movement of planting material since the disease is only partially systemic and wilt symptoms are not observed. Maiden suckers or followers from infected plants remain disease-free when planted in isolation (Soguilon et al. 1994 b). It is not known how the disease has spread so widely in the Philippines, from Mindanao in the south to Luzon in the north, and in the absence of data, suggestions about the means of spread are speculative. A plausible hypothesis is that insect or mechanical transmission were involved. Aerial, transoceanic spread of Moko bacterial wilt by insects is not known (Sequeira 1958) or insects.

Debudding

Removal of the male bud is an accepted measure that prevents insect transmission of Moko bacterial wilt on Bluggoe (French and Sequeira 1968, Lehmman-Danzinger 1987, Ploetz et al. 2003, Stover 1972, 1993, Thwaites et al. 2000). Debudding experiments showed that infection did not occur when the bud was broken off before the first row of male flowers was exposed (Buddenhagen and Elsasser 1962). Early work on Bugtok disease had suggested that insect transmission occurred through both male and female flowers, leading to the tentative conclusion that debudding would not be effective to control Bugtok on 'Saba' and 'Cardaba' (Eden-Green and Seal 1993, Eden-Green 1994, Roperos and Magnaye 1991, Soguilon 1990). Later textbooks also stated that removing the male bud was not effective in controlling the spread of the disease (Jeger et al. 1995, Ploetz et al. 2003, Thwaites et al. 2000) on the premise that infection may also occur through the female flowers. Molina (1996) compared debudding, bagging of the inflorescence, sanitation, and tool disinfection as measures to control Bugtok in the Visayan islands of the Philippines. All the measures significantly reduced disease incidence. Farmers found bagging to be impractical because of the height of the plants. Sanitation and early debudding reduced infection from an initial incidence of 88% to 6% after 12 months. The work of Molina (1996) suggests that the recommendations for controlling Moko bacterial wilt should be extended to Bugtok disease.

References


Screening banana cultivars for resistance to bacterial xanthomonas wilt

G. Welde Michael, K. Bobosha, G. Blomme, T. Addis, S. Mekonnen and T. Mengesha

In Ethiopia, bananas are cultivated together with enset at altitudes between 1050 and 2100 m above sea level. Both are hosts to the bacterial wilt pathogen Xanthomonas campestris pv. musacearum (Xcm) (Yirgou and Bradbury 1968, 1974). Since enset plants are harvested before flowering, insect vector transmission from flower to flower is not an issue. Insect vector transmission is rarely observed on bananas on plantains and bananas. Pp.130-152. In Seminar proceedings. Improving citrus and banana production in the Caribbean through phytosanitation, 2-5 September 1986, St. Lucia, WI. CTA/CARDI, Wageningen, the Netherlands.


grown above 1700 masl, but it occurs at lower elevations.

The commonly grown banana cultivars are: ‘Pisang awak’ (ABB), several ‘Cavendish’ cultivars (AAA), ‘Uganda red’ (AAA) and East African highland bananas (AAA-EAHB). It was observed that all these banana cultivars can develop the disease, bacterial xanthomonas wilt (BXW), after being infected by contaminated tools. Finding resistant banana cultivars would be a long-term and cost-effective solution. The objective of this study was to assess local and exotic banana cultivars for resistance to enset bacterial wilt under artificial inoculation conditions.

Materials and methods
Forty banana cultivars (Table 1) obtained from the Melkasa Agricultural Research Center, Melkasa, Ethiopia were screened for resistance to BXW one year after planting in an experimental field at the Awassa Agricultural Research Center, Awassa, Ethiopia.

Five sword suckers of each cultivar were field-established and plant spacing was 2.5 m between plants in a row and 3 m between the rows. The 5 plants of a specific genotype were planted in a single row. One year after planting, 4 motherplants per cultivar were inoculated with 3 ml of a virulent Xcm isolate suspension whose cell concentration was adjusted to 1x10^6 cfu/ml. The Xcm isolate was collected from Hagere Selam, southern Ethiopia (Quimio 1994). The motherplants were inoculated at the base of the petioles of the first two expanded leaves using a 10 ml hypodermic syringe with needle. The single control plant in each row was inoculated with the same volume of sterile distilled water. Data were collected on motherplants 7, 15, 21, 30, 45, 60, 75, 90 and 120 days after inoculation.

Results and discussion
All the inoculated banana cultivars developed disease symptoms within 45 to 120 days (and 94% within 75 days) of inoculation, except for one plant of the cultivar ‘Kamaramasenge’ (Table 1). Some of the un-inoculated control plants also developed typical bacterial wilt symptoms, presumably due to the natural spread of the disease. Although the inoculation method used was artificial and could mask differences in susceptibility, particularly to infection via inflorescences, the trial showed that none of the banana cultivars was immune to infection by Xcm.

References
Effect of combined inoculations of endophytic fungi on the biocontrol of *Radopholus similis*

A. zum Felde, L.E. Pocasangre, C.A. Carñizares Monteros, R.A. Sikora, F.E. Rosales and A.S. Riveros

Of the various plant-parasitic nematodes affecting bananas and plantain worldwide, *Radopholus similis* is recognised as the most important (Gowen et al. 2005). Damage caused by *R. similis* begins with tunnels of necrotic tissue in roots and corms, which affect water and nutrient uptake thereby lengthening the growing period. Eventually, roots rot due to secondary infection of damaged tissue by bacteria and fungi, leading to the toppling of banana plants as a result of root destruction and loss of anchorage (Gowen et al. 2005, Sarah et al. 1996). *R. similis* migrates from necrotic root tissue to adjoining fresh tissue and through the soil to gain access to non-infested tissue from the same plant or another plant (Sarah et al. 1996). Substantial yield gains of between 20% and 75% have been demonstrated following the application nematicides to control *R. similis* and nematodes in general (Broadley 1979, McSorley and Parrado 1986, Sarah 1989, Gowen 1994).

In commercial banana plantations of Latin America, nematode control basically relies on the use of granular organophosphate and carbamate nematicides (Marín 2005). Cultural practices, such as the use of organic amendments, crop rotations, fallows and clean planting material are also used, but with varying success. Some biocontrol products, which contain bacteria, such as Blue Circle™ (*Burkholderia cepacia*), a fungus, such as Paecil™ (*Paecilomyces lilacinus*), or the killed fermentation products of a fungus, such as DiTera™ (*Myrothecium verrucaria*), are available for nematode management (APS Biological Control Committee 2005), but banana producers do not generally use them because of a lack of adequate control.

To improve the activity and thereby increase the options for the biological management of *R. similis* in banana, novel
biological control agents, such as endophytic fungi from promising locations, are being studied for field application. Promising locations include areas in commercial plantations where nematicides are not used and nematode sampling has revealed low nematode densities over extended periods of time - such as parts of the Motagua Valley in Guatemala (zum Felde et al. 2005) - and organic plantations and alternative production areas, where bananas and plantain are grown alongside other crops, such as cacao and timber species (Meneses et al. 2003). Clay (1989) first suggested the potential of endophytes from the endosphere as biocontrol agents of insect pests. More recently, fungal endophytes from the endorhiza have been identified as biocontrol agents in bananas (Pocasangre et al. 2000, Carñizares Monteros 2003, Niere et al. 2004, Vu et al. 2004, zum Felde et al. 2005), vegetables (Hallmann et al. 2001), rice (Padgham et al. 2005, Padgham and Sikora 2006) and maize (Wicklow et al. 2005).

In an attempt to improve the stability, intensity and/or reliability of the biocontrol method, numerous authors have studied the effect of combining biocontrol agents (review by Meyer and Roberts 2002). Combinations are not always beneficial, as antagonism can occur between biocontrol organisms, and lead to unchanged control levels (Zaki and Maqbool 1991, Viena and Abanoy 2000) or even to lower control (Esnard et al. 1998, Chen et al. 2000), when compared to individual applications of biocontrol agents. However, many of the combinations studied have resulted in increased biocontrol levels (Guetsky et al. 2001, Guetsky et al. 2002, Meyer and Roberts 2002). Combinations of biocontrol agents assessed against nematodes include fungi with fungi (Khan et al. 1997, Duponnois et al. 1998, Hojat Jalali et al. 1998, Chen et al. 2000) and fungi with bacteria (Maheswari and Mani 1988, de Leij et al. 1992, Siddiqui and Mahmood 1993, Perveen et al. 1998, Chen et al. 2000), with most combinations involving two organisms, but few involving combinations of three or more organisms (Esnard et al. 1998).

The majority of biocontrol agents that have been assessed in combinations against nematodes were isolated from the rhizosphere or rhizoplane and tested on Meloidogyne spp. (Meyer and Roberts 2002). Diedhiou et al. (2003) assessed an arbuscular mycorrhizal fungi (AMF), Glomus coronatum, and a non-pathogenic endophytic Fusarium oxysporum against Meloidogyne incognita in tomato. They obtained interesting results regarding the interaction of the two fungi within the plant, but observed no increased nematode control related to combined inoculation. Sikora and Reimann (2004), who worked with the plant health promoting rhizobacterium Rhizobium etli G12, the AMF Glomus intraradices and a rhizobacterium associated with AMF spores, found that in long-term experiments, rhizobacteria in combination with G. intraradices reduced gall and egg mass production of M. incognita. To the best of our knowledge, studies combining two or more endophytic fungi have not yet been undertaken.

The objective of the study was to evaluate the effect of combined inoculations of endophytic fungi on R. similis management in banana roots.

Materials and methods

The fungi used in the study were isolated from healthy banana and plantain root material, collected in the Motagua Valley, Guatemala (zum Felde et al. 2005) and the Talamanca and Sixaola regions of Costa Rica (Meneses et al. 2003, Carñizares Monteros 2003). All isolates were identified as R. similis-antagonistic fungi through in vitro and in vivo screening tests carried out at the nematology and phytopathology laboratories at CATIE, Turrialba, Costa Rica, during the period from January 2002 to December 2003 (zum Felde et al. 2005, Meneses et al. 2003, Carñizares Monteros 2003). The most effective isolates were identified to species level by Dr. H. Nierenberg, at the Biologische Bundesanstalt, in Berlin, Germany. The non-pathogenic nature of all R. similis-antagonistic fungi was established by pathogenicity tests in planta.

In 2005, the vegetative compatibility of all the F. oxysporum isolates were tested against 56 reference strains of pathogenic F. oxysporum isolates (F. oxysporum f. sp. cubense, radicis-lycopersici and lycopersici), in Bonn, Germany (A. zum Felde and T. Vu Thi Thanh, unpublished data).

The four endophytes that had provided greatest nematode control in the in planta experiments, identified as F. oxysporum and T. atroviride isolates, were used for the
present study. To compare the effects of combined inoculations of these endophytic fungi on nematode management, 15-week-old tissue-culture plantlets of the cultivar ‘Williams’ (Musa AAA) were inoculated with conidia from one, two or four endophytes (Table 1). Plants were obtained from a commercial tissue-culture laboratory, and transported in multi-trays containing 200 plants in a sterile potting mix (commercial seeding substrate). Each plant was rooted in approximately 15 cm³ of potting mix. Roots in potting mix were not washed prior to inoculation, increasing the capacity of conidia to attach to the roots and be absorbed into the adjacent substrate.

The four selected isolates were grown on 100% potato dextrose agar plates for two weeks, until sufficient conidia to prepare suspensions were available. Conidia were removed from the media surface by pipetting up to 20 ml of sterile water onto the plates and gently scraping the conidia off the media surface by using a sterile metal bacterial cell spreader. The conidia were then separated from mycelium by pouring the suspension through a piece of sterile gauze into a sterile beaker. Conidia concentration was determined by using a Neubauer hemacytometer. Three 500 ml beakers, each containing 300 ml of a 1 x 10⁶ cfu/ml conidia suspension, were prepared for each isolate and used once for each type of inoculation. Dip inoculations were performed by simultaneously dipping the root ball of 11 plants into a 500 ml beaker for 5 minutes. For dual and multiple inoculations, roots were successively dipped for 5 minutes in a beaker containing a given conidia suspension.

After inoculation, plants were planted in 500 ml pots containing a sterile mix of sand and soil (1:1). Two weeks after planting, a total of 2 ml of tap water containing 500 live, vermiform R. similis nematodes was pipetted into three small holes (1 to 2 cm deep) made at the base of each banana plant. The holes were covered with surrounding soil. R. similis were taken from sterile carrot disk cultures prepared at CATIE (Speijer and Gold 1996). The nematodes originated from heavily infested banana plantations in Costa Rica. Plants were watered regularly, but no fertilizer was applied.

Two months after nematode inoculation, plants were harvested and fresh root weight recorded. Nematodes were then extracted from roots using a maceration and sieving method adapted from Speijer and De Waele (1997). Each root system was cut into pieces about 1 cm long, and macerated in a commercial blender in 200 ml of tap water for 10 seconds at low speed, and 10 seconds at high speed, with a 5-second interval between the two maceration steps. The suspension was poured through nested sieves of 1000 µm, 150 µm and 45 µm apertures. The content of the 45 µm sieve was washed into a 250 ml plastic container with a lid. Each container was filled up to 200 ml and, prior to counting, the contents were homogenized by agitating with an aquarium pump. For each sample, nematode density was determined from two sub-samples of 2 ml using a counting slide (Speijer and De Waele 1997). The experimental design was a completely randomized block design, with nine treatments and eleven replicates (n=11) per treatment. Nematode data was √(x + 0.5) transformed for analysis. Data was analysed using the SAS program (SAS/STAT® Software, SAS Institute Inc.). Means were separated using Duncan’s Multiple Range test and orthogonal contrasts.

**Results**

Two months after inoculation with R. similis, the total number of nematodes in the roots and their density were both significantly lower

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**Table 1: Treatments used to test the effect on Radopholus similis of single and combined inoculations of endophytic fungi.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Codes used for endophytes</th>
<th>Endophyte species and origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single inoculation MT-20</td>
<td>-</td>
<td>Trichoderma atroviride, from Motagua, Guatemala</td>
</tr>
<tr>
<td>Single inoculation S2</td>
<td>-</td>
<td>Trichoderma atroviride, from Sixaola, Costa Rica</td>
</tr>
<tr>
<td>Single inoculation S9</td>
<td>-</td>
<td>Fusarium oxysporum, from Sixaola, Costa Rica</td>
</tr>
<tr>
<td>Single inoculation P12</td>
<td>-</td>
<td>Fusarium oxysporum, from Talamaca, Costa Rica</td>
</tr>
<tr>
<td>Dual inoculation MT-20 &amp; S2</td>
<td>-</td>
<td>Trichoderma atroviride, Guatemala &amp; Costa Rica</td>
</tr>
<tr>
<td>Dual inoculation S9 &amp; P12</td>
<td>-</td>
<td>Fusarium oxysporum, Costa Rica</td>
</tr>
<tr>
<td>Multiple inoculation MT-20, S2, S9 &amp; P12</td>
<td>-</td>
<td>T. atroviride &amp; F. oxysporum, Guatemala &amp; Costa Rica</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

1 Only R. similis was inoculated
in the plants inoculated with endophytes than in the control plants (Table 2). Dual inoculations reduced the number, but not the density, of *R*. *similis* more than single inoculations, but the multiple inoculation did not significantly reduce numbers or densities over that obtained by dual inoculations. *Fusarium* isolates (S9 and P12) tended to suppress nematodes better than the *Trichoderma* isolates (MT-20 and S2).

Orthogonal contrasts revealed highly significant differences between the total population and density of nematodes in inoculated and control plants, as well as between plants inoculated with all four fungi or only two at a time (Table 3). The plants receiving dual inoculations had significantly different populations of *R*. *similis*, with those inoculated with the *Fusarium* isolates S9 & P12 containing less nematodes than those inoculated with the *Trichoderma* isolates MT-20 & S2, though nematode density was not significantly different. The effect of dual or single inoculations significantly differed from one another, with the greater control seen in plants inoculated with two fungi. Nematode densities were not significantly different between plants receiving single inoculations of either the *Fusarium* or the *Trichoderma* isolates.

**Discussion**

The results of our study indicate that combining compatible biocontrol agents may provide improved protection against *R*. *similis* when compared to the use of only one biocontrol agent.

We used sequential inoculations to avoid possible negative interactions between fungal conidia prior to inoculation, and stuck to our pre-established inoculation time of 5 minutes for each fungus. Experiments carried out at the University of Bonn in 1998 and 1999, involving the biocontrol with non-pathogenic *F. oxysporum* isolates of the disease caused by *F. oxysporum* f.sp. *cubense*, had established the optimum inoculation duration and conidia density for effective colonization of the root system by pathogenic and non-pathogenic strains of *F. oxysporum* (L. Pocasangre unpublished data). These experiments compared the efficiency of dip inoculations for conidia suspensions ranging from 1 x 10^2 to 1 x 10^6 cfu/ml and for dips lasting between 5 and 30 minutes revealed that a 5 min dip in a 1 x 10^6 cfu/ml suspension was optimal for root colonization of tissue culture banana plants (Pocasangre 2000).

In addition, several studies performed at CATIE over the last six years suggest that dipping the root system of tissue culture plants in a conidia suspension of at least 1 x 10^5 cfu/ml for 5 minutes is an effective inoculation system (zum Felde 2002, Canizares 2003, Meneses 2003, Pocasangre *et al.* 2004). The concentration of the conidia suspension played a more important role in effective root colonization by the fungi, than the duration of the dip inoculation. Desai and Dange (2003) came to the same conclusions regarding the relationship between successful colonization and inoculum concentration and dip duration. In their work with castor bean wilt (*F. oxysporum* f.sp. *ricinum*), they found a positive correlation between wilt incidence (fungal colonization of the plant tissues) and increased inoculum concentration, but none with root dipping time (Desai and Dange 2003). Although many studies have used dip inoculations to apply both pathogenic and biocontrol fungi and bacteria to plant roots, few authors give details on the procedure. Often, only the concentration of inoculum is given, while the length of the dip is omitted.

**Table 2: Effect of endophytic fungi on *Radopholus similis* number and density in banana roots, eight weeks after inoculation (n=11).**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of <em>R. similis</em> in root system</th>
<th>% reduction</th>
<th>Density of <em>R. similis</em> (number/g of root)</th>
<th>% reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single (MT-20)</td>
<td>2582</td>
<td>b</td>
<td>44</td>
<td>480</td>
</tr>
<tr>
<td>Single (S2)</td>
<td>2327</td>
<td>bc</td>
<td>49</td>
<td>473</td>
</tr>
<tr>
<td>Single (P12)</td>
<td>2173</td>
<td>bc</td>
<td>53</td>
<td>661</td>
</tr>
<tr>
<td>Single (S9)</td>
<td>1964</td>
<td>cde</td>
<td>57</td>
<td>469</td>
</tr>
<tr>
<td>Dual (MT-20 &amp; S2)</td>
<td>1800</td>
<td>ed</td>
<td>61</td>
<td>337</td>
</tr>
<tr>
<td>Dual (S9 &amp; P12)</td>
<td>1691</td>
<td>e</td>
<td>63</td>
<td>301</td>
</tr>
<tr>
<td>Multiple (MT-20, S2, S9 &amp; P12)</td>
<td>1600</td>
<td>e</td>
<td>65</td>
<td>245</td>
</tr>
<tr>
<td>Control</td>
<td>4582</td>
<td>a</td>
<td>-</td>
<td>1721</td>
</tr>
</tbody>
</table>

Means in columns followed by different letters are significantly different at p<0.05 according to Duncan’s Multiple Range Test.

Data were √(x + 0.5) transformed for statistical analysis, while means presented are those of original data.

**Table 3: Orthogonal contrasts carried out on *Radopholus similis* population and density data collected eight weeks after inoculation.**

<table>
<thead>
<tr>
<th>Contrasts</th>
<th>Number of <em>R. similis</em> in root system</th>
<th>Density of <em>R. similis</em> (number/g of root)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control vs Treatments with fungi</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Multiple vs Dual inoculations</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>MT-20 &amp; S2 vs S9 &amp; P12</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>S9 &amp; P12 vs S9, P12</td>
<td>*</td>
<td>n.s.</td>
</tr>
<tr>
<td>MT-20 &amp; S2 vs MT-20, S2</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>MT-20 vs S2</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Significantly different at p<0.05* or p<0.01**, n.s.: not significant.
Consequently, we do not think that increasing the total inoculation time affected fungal colonization of roots by individual fungi, as the conidia of each fungus were only in contact with the root system for 5 minutes, giving each fungus equal opportunity to adhere to roots. The total amount of conidia adhering and later colonizing roots is probably greater in plants inoculated with two or all four fungi, but that was the goal of the experiment - to observe the effects of combined inoculations, as opposed to a simple one. We do not believe that the lower number of nematodes in the roots of inoculated plants with more than one fungal isolate can solely be attributed to an increased inoculum charge. We think it is related to the synergistic effects of these compatible biocontrol agents.

Guetsky et al. (2002) demonstrated that the use of a combination of biocontrol agents improved biocontrol efficacy and consistency. Meyer and Roberts (2002) suggest that more effective disease suppression using combinations of biocontrol agents is due to additive or synergistic effects of their combined mechanisms. While the exact modes of action against R. similis need to be confirmed, they may differ between isolates or genus. In screening tests conducted prior to in planta testing of isolates, both Trichoderma isolates (MT-20 & S2) appeared to parasitize R. similis in vitro (zum Felde 2002, Carrizares Monerons 2003), while the metabolites of the two Fusarium isolates (P12 & S9) applied in vitro had nematostatic and nematicidal effects on R. similis (Carrizares Monerons 2003, Menenses Hérnandez 2003).

Guetsky et al. (2001) postulated that as long as biocontrol agents have different ecological requirements, combinations of agents with different requirements will likely increase reliability and decrease variability of biocontrol. Meyer and Roberts (2002) conclude that negative effects of combinations of biocontrol agents result from their mechanism(s) of control being directed not only at the plant pathogen, but also at the companion biocontrol agent within the combination. Indeed, Trichoderma and Fusarium spp. have both been successfully used to suppress Fusarium wilt (Park et al. 1988, Mao et al. 1998). However, even though all four tested biocontrol agents were isolated from internal tissues of banana roots, and as such presumably occupy the same or similar ecological niches, they nevertheless appear not to compete with each other and may even complement each other. In addition, treating seedlings with these endophytes is a very practical and economically viable approach to biocontrol, compared to treating soil. Field trials to evaluate the effects of the four individual isolates are presently underway, with promising initial results.

As the results of the present study reveal greater biocontrol when isolates are use in combination and suggest a more stable biocontrol system, further field trials are necessary to fully confirm their effectiveness and potential in the field. Further research is also needed to explore the mechanisms by which the fungal endophytes control R. similis in banana root tissues and their movement or transfer from one plant generation to the next.

Acknowledgements

The authors would like to thank the members of the Nematology and Soil Health Unit at CATIE, Turrialba, Costa Rica for their support and help in executing the present study, and the DAAD (German Academic Exchange Service) and INIBAP-LAC for funding the researchers.

References


Root system and shoot growth of banana (Musa spp.) in two agro-ecological zones in Nigeria

G. Blomme, R. Swennen, R. Ortiz and A. Tenkouano

The concept of phenotypic plasticity of roots refers to the ability of cultivars to adapt their root structure to changes in the environment (O’Toole and Bland 1987, Draye 2002). The most significant parameter influencing root growth and development is, by far, the edaphic environment which, for example, includes soil temperature, moisture level, partial pressure of carbon dioxide and oxygen, and nutrient availability. Ambient factors such as air temperature, day length, light intensity and partial pressure of carbon dioxide affect the provision of nutrients and growth regulators from the shoot to the root system. Environmental factors also influence the shoot/root ratio of a plant (Wright 1976, Jung 1978, Smucker 1984, Bastow Wilson 1988, Kasperbauer 1990, Squire 1993, Martinez Garnica 1997, McMichael and Burke 1998). Research on the development of the cotton (Gossypium hirsutum L.) root system has shown that considerable changes can occur under various soil conditions (Pearson 1965, Adams et al. 1967, Halevy 1976), while Bennie and du T. Burger (1981) reported that increased mechanical impedance (i.e. increased soil bulk density) reduced root elongation in maize (Zea mays L.), wheat (Triticum aestivum L.) and groundnut (Arachis hypogaea L.).

The positive effect of increased soil porosity on root growth and development has been demonstrated for dessert bananas (AAA) (Sioussaram 1968, Champion and Sioussaram 1970, Delvaux and Guoy 1989). In addition, there is a significant effect of climatic conditions on dessert banana root and shoot growth (Robin and Champion 1962, Turner 1970, Turner and Lahav 1983). Robinson and Alberts (1989) reported that root growth is slower at lower temperatures. For example, in the Cavendish variety ‘Williams’ (AAA), axis extension was nearly 3 cm per day at 25°C, below 0.5 cm per day at 15°C and ceased at 11.5°C.

Beugnon and Champion (1966) reported an effect of planting date on root and shoot growth for the dessert banana ‘Poyo’ (AAA). In plantains (AAB), Irizarry et al. (1981) reported substantial influence of soil type on plant growth, root system development and distribution. They demonstrated that improved knowledge on root system distribution could lead to more effective cultural practices such as irrigation and fertilization. However, their study was
carried out for only one plantain cultivar (‘Maricongo’). Information on root system growth and development across different locations is lacking for a wide range of genotypes. Hence, this study was designed to determine agro-ecological effects on root system development, corm and aerial growth traits of a wide range of *Musa* spp. genotypes.

**Materials and methods**

Seventeen *Musa* spp. genotypes (Table 1) were evaluated in two agro-ecological zones of Nigeria: the humid forest and moist savannah. The humid forest was at the International Institute of Tropical Agriculture (IITA) High Rainfall station at Onne in southeastern Nigeria (4°42’ N, 7°10’ E, 10 m above sea level). The soil is derived from coastal sediments and is a deep and freely drained Typic Paleudult/Haplic Acrisol (FAO/ISRIC/ISSS 1998). This soil belongs to the coarse-loamy, siliceous iso-hyperthermic family. The average annual rainfall is 2400 mm distributed monomodally from February to November. The average daily solar radiation is 12.6 MJ/m². Details of the site are described in Ortiz et al. (1997).

The moist savannah was at the IITA Abuja station in central Nigeria (9°16’ N, 7°20’ E, 300 masl). The soil is a luvisol/Lixisol high in nutrients but poorly drained, with a pH of 6. The average annual rainfall is 1300 mm distributed unimodally from April to October. The average daily solar radiation is 16.02 MJ/m². Soil chemical analysis was carried out at both locations (Table 2).

The planting material was produced by tissue culture (Vuylsteke 1989, 1998). The plantlets were acclimatized for six weeks in a greenhouse nursery (Vuylsteke and Talengera 1998, Vuylsteke 1998) at Onne, prior to transplantation in the field at Onne in May and Abuja in August.

Each genotype was represented by four and three plants in Onne and Abuja, respectively. The field layout in Onne was a randomized complete block design with two replications of two plants per genotype, while a completely randomized field design was used at Abuja. Plant spacing was 4 m x 4 m at both locations. The experimental area was treated with the nematicide Nemacur (a.i. fenamiphos) at a rate of 15 g/plant (3 treatments) to reduce nematode numbers. The field was fertilized with muriate of potassium (a.i. K20, 60% K) at a rate of 600 g plant⁻¹ year⁻¹, and urea (47% N) at a rate of 300 g plant⁻¹ year⁻¹, split into six equal applications during the rainy season. No

### Table 1. Genotypes evaluated.

<table>
<thead>
<tr>
<th>Name</th>
<th>Genomic group</th>
<th>Ploidy</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yangambi km 5</td>
<td>AAA</td>
<td>3</td>
<td>Dessert banana</td>
</tr>
<tr>
<td>Valery</td>
<td>AAA</td>
<td>3</td>
<td>Dessert banana</td>
</tr>
<tr>
<td>Pisang Ceylan</td>
<td>AAB</td>
<td>3</td>
<td>Dessert banana (Mysore)</td>
</tr>
<tr>
<td>Obino ewai</td>
<td>AAB</td>
<td>3</td>
<td>Plantain</td>
</tr>
<tr>
<td>Pelitipa</td>
<td>ABB</td>
<td>3</td>
<td>Cooking banana</td>
</tr>
<tr>
<td>Cardaba</td>
<td>ABB</td>
<td>3</td>
<td>Cooking banana</td>
</tr>
<tr>
<td>Fougamou</td>
<td>ABB</td>
<td>3</td>
<td>Cooking banana</td>
</tr>
<tr>
<td>TMP 3x 15108-6</td>
<td>AAB x AA</td>
<td>3</td>
<td>Secondary triploid (Bobby tannap x Calcutta 4) x SH-3362</td>
</tr>
<tr>
<td>TMP 4x 2796-5</td>
<td>AAB x AA</td>
<td>4</td>
<td>Plantain hybrid (Bobby tannap x Pisang lilin)</td>
</tr>
<tr>
<td>TMP 4x 7152-2</td>
<td>AAB x AA</td>
<td>4</td>
<td>Plantain hybrid (Obino ewai x Calcutta 4)</td>
</tr>
<tr>
<td>TMP 4x 548-9</td>
<td>AAB x AA</td>
<td>4</td>
<td>Cooking banana hybrid (SH-3386 x SH-3320)</td>
</tr>
<tr>
<td>FHIA-03</td>
<td>AAB x AA</td>
<td>4</td>
<td>AVP-67 x SH-3142</td>
</tr>
<tr>
<td>SH-3640</td>
<td>AAB x AA</td>
<td>4</td>
<td>Prata Anã x SH-3393</td>
</tr>
<tr>
<td>SH-3436-9</td>
<td>AAA x AA</td>
<td>4</td>
<td>Somaclonal variant of SH-3436 (Highgate (Gros Michel) x SH-3142)</td>
</tr>
<tr>
<td>EMB 402</td>
<td>AAB x AA</td>
<td>4</td>
<td>Pacovan x Calcutta 4</td>
</tr>
<tr>
<td>EMB 403</td>
<td>AAB x AA</td>
<td>4</td>
<td>Prata anã x Calcutta 4</td>
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</table>

### Table 2. Chemical analysis of top soil at Onne and Abuja.

<table>
<thead>
<tr>
<th></th>
<th>Onne</th>
<th>Abuja</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0-15 cm</td>
<td>15-30 cm</td>
</tr>
<tr>
<td>pH H2O (1:1)</td>
<td>4.2</td>
<td>4.3</td>
</tr>
<tr>
<td>Org C %</td>
<td>1.20</td>
<td>0.78</td>
</tr>
<tr>
<td>Kjel N %</td>
<td>0.11</td>
<td>0.06</td>
</tr>
<tr>
<td>Bray-P (mg/kg)</td>
<td>44.05</td>
<td>62.55</td>
</tr>
<tr>
<td>Sand %</td>
<td>79</td>
<td>73</td>
</tr>
<tr>
<td>Silt %</td>
<td>17</td>
<td>5</td>
</tr>
<tr>
<td>Clay %</td>
<td>14</td>
<td>22</td>
</tr>
<tr>
<td>Exch Ca (cmol/kg)</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Exch Mg (cmol/kg)</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Exch K (cmol/kg)</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Exch Na (cmol/kg)</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>ECEC (cmol/kg)</td>
<td>2.5</td>
<td>2.2</td>
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Table 3. Expected mean squares for various traits assessed at flower emergence in Onne and Abuja.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Type III Expected Mean Square</th>
<th>df</th>
<th>LA (cm²)</th>
<th>NL</th>
<th>PH (cm)</th>
<th>PC</th>
<th>CW (g)</th>
<th>CH (cm)</th>
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<tbody>
<tr>
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<td>Var(Error) + 72.25 Var(LOC)</td>
<td>1</td>
<td>36612513003***</td>
<td>12</td>
<td>83971***</td>
<td>6625***</td>
<td>411995870***</td>
<td>785***</td>
</tr>
<tr>
<td></td>
<td>+ Q(LOC*GEN)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotype</td>
<td>Var(Error) + Q(ngen,LOC*GEN)</td>
<td>16</td>
<td>3957624450***</td>
<td>57**</td>
<td>10152***</td>
<td>564***</td>
<td>27024642***</td>
<td>69***</td>
</tr>
<tr>
<td>Genotype x location</td>
<td>Var(Error) + Q(LOC*GEN)</td>
<td>16</td>
<td>2865643023***</td>
<td>45**</td>
<td>1787***</td>
<td>180***</td>
<td>13436037***</td>
<td>26***</td>
</tr>
<tr>
<td>Residual</td>
<td></td>
<td>76</td>
<td>623876137</td>
<td>6</td>
<td>686</td>
<td>41</td>
<td>1533972</td>
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<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Type III Expected Mean Square</th>
<th>df</th>
<th>WW (cm)</th>
<th>DR (g)</th>
<th>NR</th>
<th>LR (cm)</th>
<th>AD (mm)</th>
<th>TL (cm)</th>
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<tbody>
<tr>
<td>Location</td>
<td>Var(Error) + 72.25 Var(LOC)</td>
<td>1</td>
<td>479***</td>
<td>212904***</td>
<td>52325***</td>
<td>1526691</td>
<td>0.13</td>
<td>787341</td>
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<td></td>
<td>+ Q(LOC*GEN)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotype</td>
<td>Var(Error) + Q(ngen,LOC*GEN)</td>
<td>16</td>
<td>51***</td>
<td>39992***</td>
<td>8645***</td>
<td>13813739***</td>
<td>1.38***</td>
<td>53000480***</td>
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<tr>
<td>Genotype x location</td>
<td>Var(Error) + Q(LOC*GEN)</td>
<td>16</td>
<td>14***</td>
<td>16464***</td>
<td>5947***</td>
<td>9830888***</td>
<td>0.33**</td>
<td>25821243*</td>
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<td></td>
<td>76</td>
<td>2</td>
<td>4035</td>
<td></td>
<td>1783</td>
<td>2826558</td>
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<table>
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<th>Source of variation</th>
<th>Type III Expected Mean Square</th>
<th>df</th>
<th>TD (g)</th>
<th>NS</th>
<th>HS (cm)</th>
<th>DFE</th>
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<tbody>
<tr>
<td>Location</td>
<td>Var(Error) + 72.25 Var(LOC)</td>
<td>1</td>
<td>351079***</td>
<td>732***</td>
<td>144251***</td>
<td>689877***</td>
</tr>
<tr>
<td></td>
<td>+ Q(LOC*GEN)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotype</td>
<td>Var(Error) + Q(ngen,LOC*GEN)</td>
<td>16</td>
<td>115615***</td>
<td>93***</td>
<td>23799***</td>
<td>33782***</td>
</tr>
<tr>
<td>Genotype x location</td>
<td>Var(Error) + Q(LOC*GEN)</td>
<td>16</td>
<td>40021***</td>
<td>22***</td>
<td>6639***</td>
<td>10932***</td>
</tr>
<tr>
<td>Residual</td>
<td></td>
<td>76</td>
<td>9061</td>
<td>4</td>
<td>1114</td>
<td>3097</td>
</tr>
</tbody>
</table>

LA: leaf area, NL: number of leaves, PH: plant height, PC: pseudostem circumference at soil level, CW: corm dry weight, CH: corm height, WW: corm widest width, DR: root dry weight, NR: number of adventitious roots or cord roots, LR: total cord root length of the mother plant, AD: average diameter at the base of the cord roots, TL: total cord root length of the mat, TD: root dry weight of the mat; NS: number of suckers, HS: height of tallest sucker, DFE: days to flower emergence

* ** *** Significant at P<0.05, 0.01 and 0.001, respectively.

df: degrees of freedom.

mulch was applied. The fungicide Bayfidan (a.i. triadimenol) was applied three times a year at a rate of 3.6 ml/plant to control black leaf streak disease, which is caused by Mycosphaerella fijiensis Morelet.

In the moist savannah zone, Musa plants can only be cultivated along riverbeds in order to survive the dry season. Plants in Abuja were therefore irrigated during the 6-month dry season at a rate of 100 mm/month. In Onne, the plants were irrigated at the same rate during the short 3-month dry season.

Plants were excavated at flower emergence and the traits measured on the mother plant were leaf area, number of leaves, plant height (from soil level to the point where the two highest petioles meet each other) and pseudostem circumference at soil level. Leaf area was calculated according to Obiefuna and Ndbizu (1979). Traits measured on the corm of the mother plant were fresh weight, height and widest width. Traits measured on the roots of the mother plant included root dry weight, the number of adventitious (cord) roots, their total length using the line intersect method (Newman 1966, Tennant 1975) and their average basal diameter measured with a Vernier calliper. Other traits were total root dry weight of the mat (i.e. mother plant and suckers) and total cord root length of the mat. All suckers were left to grow. The number of suckers on the corm and the height of the tallest sucker were also noted, as well as the number of days from field planting to flower emergence.

Statistical analysis was performed using the SAS package (SAS 1989). A 3-way ANOVA was carried out to determine the effect on the different traits of location, genotype and genotype x location interaction. Location was considered as random and genotype as fixed. Means were separated by the pair-wise comparison t-test of Least Square Means. Linear correlation analysis was carried out between the same plant traits at both locations.

Results

The effect of location was significant on most aerial growth, corm and root system traits, except for the number of leaves, total cord root length of the mother plant and mat, and average cord root diameter of the mother plant (Table 3). The genotype x location interaction was significant for all measured traits.

The plants in Abuja had a significantly larger leaf area, a significantly taller and bigger pseudostem and a bigger corm (Table 4). The larger corms of plants in Abuja were associated with a significantly higher number of cord roots. However, the total cord root length was similar in both locations (Table 4). Cord roots were on average
shorter in Abuja. Indeed, during excavation, it was observed that the longest cord roots in Abuja never extended more than 1.5 m from the corm, while cord roots at Onne could extend up to 3 m from the corm. A higher number of first and second order lateral roots were observed for plants growing at Onne (however no detailed assessment was done on these lateral roots). The leaf area/cord root length ratio and the leaf area/root dry weight ratio were significantly higher in Abuja than in Onne, indicating that the plants in Onne had a relatively better developed root system (Table 4).

More suckers per mat were observed at Abuja (Table 4), most probably because of the larger corm size, which could also explain the higher number of roots observed in Abuja. Despite this increased competition between suckers, the mean height of the tallest sucker at flower emergence was significantly taller in Abuja (Table 4). The tallest suckers in Abuja reached 64% of the height of the flowering mother plant compared to 48% in Onne. The number of days to flowering was significantly higher in Abuja (Table 4). The mean height of the flowering mother plant in Abuja never extended more than 1.5 m from the corm, while cord roots at Onne could extend up to 3 m from the corm. A higher number of first and second order lateral roots were observed for plants growing at Onne (however no detailed assessment was done on these lateral roots). The leaf area/cord root length ratio and the leaf area/root dry weight ratio were significantly higher in Abuja than in Onne, indicating that the plants in Onne had a relatively better developed root system (Table 4).

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Significant correlations between locations were found for number of leaves, plant height, plant circumference, corm traits, average cord root diameter, number of suckers, height of the tallest sucker and days to flowering (Table 5). No correlation was found between leaf area and other root system traits, which could suggest modifications in allocation patterns or different mortality rates according to the locality.

Discussion

The significant effect of location on most growth traits is in accordance with previous reports from Wright (1976), Jung (1978) and Kasperbauer (1990) who stated, for other crops, that environmental conditions may interact with the genetic character of plants.

The shorter cord roots suggested by the higher number of roots observed on the plants grown in Abuja, for the same total root length in both locations, could reflect reduced growth as a result of the higher percentage of clay (28 % at Abuja compared to 18 % at Onne) and silt (36 % at Abuja compared to 6 % at Onne). Irizarry et al. (1981) reported, for a plantain cultivar, a significant reduction in cord root length in heavier soil types.

But if mechanical impedance had an effect on the average length of cord roots, it did not have an effect on their average diameter, contrary to reports that have shown a correlation between high impedance and thicker roots of maize (Bennie 1979, Boone and Veen 1982, Shierlaw and Alston 1984), wheat (Bennie 1979, Collis-George and Yogananth 1985), cotton (Bennie 1979) and potatoes (Solanum tuberosum L.) (Boone et al. 1985). We observed no effect of the type of soil on average cord root diameter, but we cannot exclude the possibility that differences in root length could be attributed to different allocation patterns or rates of mortality in each locality.

Table 4. Mean values and standard error of various growth traits measured on plants grown at Onne and Abuja.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Onne ±SE</th>
<th>Abuja ±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA (cm²)</td>
<td>89 088.7 ±3 438.9</td>
<td>126 231.3 ±6 730.8</td>
</tr>
<tr>
<td>NL</td>
<td>12.0 ±0.5</td>
<td>12.7 ±0.6</td>
</tr>
<tr>
<td>PH (cm)</td>
<td>241.3 ±4.4</td>
<td>298.7 ±8.6</td>
</tr>
<tr>
<td>PC (cm)</td>
<td>63.2 ±0.9</td>
<td>79.1 ±2.2</td>
</tr>
<tr>
<td>CW (g)</td>
<td>5 064.2 ±159.6</td>
<td>9 005.5 ±508.8</td>
</tr>
<tr>
<td>CH (cm)</td>
<td>20.4 ±0.4</td>
<td>26.0 ±0.8</td>
</tr>
<tr>
<td>WW (cm)</td>
<td>19.7 ±0.3</td>
<td>23.9 ±0.6</td>
</tr>
<tr>
<td>DR (g)</td>
<td>321.0 ±14.6</td>
<td>230.0 ±14.1</td>
</tr>
<tr>
<td>NR</td>
<td>151.7 ±5.4</td>
<td>198.1 ±10.5</td>
</tr>
<tr>
<td>LR (cm)</td>
<td>6 353.7 ±315.3</td>
<td>6 206.7 ±328.8</td>
</tr>
<tr>
<td>AD (mm)</td>
<td>5.7 ±0.1</td>
<td>5.7 ±0.1</td>
</tr>
<tr>
<td>TL (cm)</td>
<td>11 120.4 ±621.3</td>
<td>11 161.6 ±602.5</td>
</tr>
<tr>
<td>TD (g)</td>
<td>511.0 ±23.6</td>
<td>399.2 ±23.7</td>
</tr>
<tr>
<td>HS (cm)</td>
<td>115.0 ±8.2</td>
<td>191.3 ±11.9</td>
</tr>
<tr>
<td>WDE (g)</td>
<td>328.7 ±7.3</td>
<td>405.3 ±17.8</td>
</tr>
<tr>
<td>LA/LR</td>
<td>16.2 ±1.1</td>
<td>22.2 ±1.6</td>
</tr>
<tr>
<td>LA/DR</td>
<td>312.8 ±17.8</td>
<td>607.4 ±39.4</td>
</tr>
</tbody>
</table>

Legend: see table 3.

Table 5. Correlation coefficients between Onne and Abuja stations for various growth parameters and days to flower emergence (17 genotypes)

<table>
<thead>
<tr>
<th>Trait</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA (cm²)</td>
<td>0.27</td>
</tr>
<tr>
<td>NL</td>
<td>0.54*</td>
</tr>
<tr>
<td>PH (cm)</td>
<td>0.79***</td>
</tr>
<tr>
<td>PC (cm)</td>
<td>0.71**</td>
</tr>
<tr>
<td>CW (g)</td>
<td>0.60*</td>
</tr>
<tr>
<td>CH (cm)</td>
<td>0.55*</td>
</tr>
<tr>
<td>WW (cm)</td>
<td>0.66**</td>
</tr>
<tr>
<td>DR (g)</td>
<td>0.35</td>
</tr>
<tr>
<td>NR</td>
<td>0.21</td>
</tr>
<tr>
<td>LR (cm)</td>
<td>0.16</td>
</tr>
<tr>
<td>AD (mm)</td>
<td>0.57*</td>
</tr>
<tr>
<td>TL (cm)</td>
<td>0.37</td>
</tr>
<tr>
<td>TD (g)</td>
<td>0.44</td>
</tr>
<tr>
<td>NS</td>
<td>0.59*</td>
</tr>
<tr>
<td>HS (cm)</td>
<td>0.55*</td>
</tr>
<tr>
<td>WDE (g)</td>
<td>0.62**</td>
</tr>
</tbody>
</table>

Legend: see table 3.
The fact that the root system of the Onne-grown plants was more branched may be related to a lower nutrient availability. Indeed, the roots need to explore a greater soil volume to access the necessary nutrients. In agreement with our results, Daw et al. (1999) reported an increase in specific root length (i.e. root length per gram dry weight) in young peach trees with reduced fertiliser application.

Since Abuja plants are taller and bigger, the physiological activity of the root system, rather than its size, seems to be the most important factor contributing to vigorous plant crop and sucker growth. The high nutrient levels of the Abuja soil (Table 2), the higher solar radiation in combination with a constant water supply throughout the year might have compensated for the relatively small root system, resulting in vigorous aerial growth. The lower root growth imposed by the heavy soil was more than offset by a better supply of solar energy, and water and nutrients in the shallow rooting zone. The latter confirm observations made by Gousseland (1983), who reported that plants with a healthy but small root system could still produce heavy bunches when grown on fertile soil.

Consequently, the harmful effect of an unfavourable factor can be compensated by modifying other factor, for example, increased fertiliser application under compacted conditions (Wild 1988). If the rooting medium is well aerated and constantly supplied with water and nutrients, other conditions being favourable, a reduced root system can support considerable shoot growth (Russell 1977).

The higher soil fertility of the Abuja soil combined with a 27% higher solar radiation should have resulted in a shorter production cycle. However plants in Abuja were planted 3 months later than the plants in Onne. This means that Abuja plants entered the dry season at a younger stage than the Onne plants. The combination of this younger growth stage with the lower night temperatures put the Abuja plants under a greater stress than the Onne plants, resulting in a longer production cycle.

The correlation analysis indicated that different genotypes respond in a similar way to the different environments for the number of leaves, pseudostem and corm traits, sucker development and days to flower emergence. Hence it is warranted, when evaluating varieties for agronomical or taxonomical studies, to include reference cultivars on which data has been collected under different agro-ecological conditions. This should make it possible to work with relative values (compared with the reference cultivar) so that comparison of varieties across environments becomes easier.

**Conclusion**

This study shows that both shoot and root traits of *Musa* genotypes are influenced by the agro-ecological environment. The enhanced performance of the plants in Abuja, supports studies in other crops, and indicates that the agricultural potential of fields in the humid tropics is less than in the sub-humid tropics, due to the poorer soils and lower solar radiation.

This study has also shown that, under optimum growing conditions, the size of the root system is not the determinant factor since a relatively less developed root system can still support vigorous plant growth when there is an ample supply of water, nutrients and solar energy. The data suggest that a distribution of the roots near the mat is sufficient, provided nutrient supplies are available. Banana farmers, who are restricted to their soil type and farming area, can improve plant growth by improving root functions through external inputs such as nutrients and water, provided nematodes and wind do not pose problems.

**Acknowledgements**

Financial support by the Flemish Association for Development Cooperation and Technical Assistance (VVOB: Vlaamse Vereniging voor Ontwikkelingsaanwerking en Technische Bijstand) and the Belgian Directorate General for Development Cooperation is gratefully acknowledged. The authors would like to underline the contribution of Dirk Vuylsteke to the conception of the experimental design, and to thank Miss Lynda Onyeukwu (IITA, Onne, Nigeria) for helping with the data collection and Mr. Philip Ragama (KARI-NARO, Kampala, Uganda) for helping with the statistical analysis.

**References**


Flower bud initiation and differentiation in plants of cv. Robusta (AAA) derived from suckers and from tissue-culture plantlets

L. Nalina, N. Kumar, K. Soorianathasundaram, J.S. Kennedy, V. Krishnamoorthy and M. Ganga

Banana is characterized by a process of flower bud initiation and differentiation that is unique among fruit crops. The inflorescence of the banana plant develops from a terminal meristem that emerges from the pseudostem made from overlaid leaf sheaths. The plant is perennial and the flower bud forms irrespective of the season, without any evident symptom. This presents problems for the study of the morphology and physiology of floral initiation.

To estimate the start of floral initiation in the pseudostem and the emergence of the inflorescence, histological studies were carried out. Since tissue-culture plantlets have largely replaced suckers in many places, because of their uniformity and high-yield potential (Hwang et al. 1984), the process of flower bud initiation was observed in both plants derived from tissue-culture plantlets and from suckers.

Materials and methods

The study was carried out at the Department of Fruit Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India from 2000 to 2002. Samples of banana meristem were collected periodically from plants of the cultivar ‘Robusta’ (AAA). Ten samples were collected from each type of plant and each development stage. The method described in Johansen (1960) was used to study the anatomy. The buds were killed and fixed in a solution of formalin, alcohol, acetic acid, alcohol and water (10:50:5:35). The buds were washed with 50% ethanol and then transferred to various concentrations of tertiary butyl alcohol (TBA) ranging from 60% to 100%. They were kept one hour at each concentration, followed by 12 hours in 100% TBA. The buds were then immersed in three parts of TBA and one part of molten wax for one hour; equal parts of wax and TBA for one hour; three parts of wax and one part of TBA for one hour; and pure molten wax in which they were kept overnight. If a trace of TBA was detected by smell, the samples were passed through pure molten wax one more time. After infiltration, the material was embedded in wax with a melting point between 52 and 54°C. Slices 12 µm thick were cut using a Spencer rotary microtome. The wax was removed using a mixture of xylene and alcohol. Saffranine was used for staining. The sections were mounted in a neutral synthetic medium, air dried and observed at 4x and 10x magnifications.

Results and discussion

Vegetative stage

In plants derived from tissue-culture plantlets and sucker, the growing point that produces the vegetative shoot emanates from a shallow depression in the corm. The important features observed in the development of the vegetative shoot apex are spiral leaf arrangement, the absence of lateral buds and almost no internodal growth. The apical meristem sections in tissue-culture plants exhibited vegetative structures (Figure 1) up to 170 days after planting in the field whereas those of plants derived from suckers showed vegetative structures up to 187 days after planting.

Transitional stage

With the onset of flowering, the shoot starts to elongate, peripheral growth of the new leaf becomes less pronounced and bracts form instead of leaf (Barker and Steward 1962). The flower bud primordia emerge in the axils of the bracts. The bracts do not show marked growth in their bases and the tip of the shoot apex changes shape to a pointed cone (Figure 2). In the present investigation, the transitional stage in plants derived from tissue-culture plantlets and from suckers ceased, respectively, 14 and 16 days after the vegetative stage.

Reproductive stage reported

Ram et al. (1962) reported that the tunica consists of three layers above the central
dome and bract primordia arise high on the flanks of the apex. The bracts have in each axil a tangentially extended, crescent-shaped meristematic, cushion-like body i.e. the floral hand, from which the flower differentiates simultaneously in double rows, alternately arranged (Figure 3). The number of days from the initiation of the reproductive phase to the formation of spathes was 23 and 25 in plants derived from tissue-culture plantlets and from suckers respectively.

The early development observed in tissue-culture plants might be due to an optimum supply of nutrients and plant hormones during their development in a culture medium. Moreover, as tissue-culture plants have a well developed root system at the time of planting, they do not expand as much energy in root formation as suckers. The tissue-culture plants of ‘Robusta’ were 21 days ahead of the plants derived from suckers with respect to shooting. Early flowering in tissue-culture plants had been showed in bananas (Swennen and De Langhe 1985, Drew and Smith 1990, Shakila 2000).

References

L. Nalina, K. Soorianathasundaram and M. Ganga work at the Department of Fruit Crops, Horticultural college and Research Institute Tamil Nadu Agricultural University, Coimbatore-641003, Tamil Nadu, India, N. Kumar at the Horticultural College and Research Institute, Tamil Nadu Agricultural University, Periyakulam-625604, Tamil Nadu, India, J.S. Kennedy at the Department of Entomology, Tamil Nadu Agricultural University, Coimbatore-641003, Tamil Nadu, India, and V. Krishnamoorthy at the Agricultural College & Research Institute, Tamil Nadu Agricultural University, Trichy-620009, Tamil Nadu, India.
Morphological characterization of two somaclonal variants of FHIA-21


Genetic improvement by crossing plantain and banana clones, which are usually infertile triploids that produce parthenocarpic fruit, is extremely difficult. For this reason, breeding by other means also plays a role in these crops (Donini and Sonnino 1998).

When Larkin and Scoweroft (1981) proposed the principle of somaclonal variability, they generated great expectations, and many tissue-culture laboratories began working on somaclonal variation. Somaclonal variants obtained through micropropagation have been reported for bananas of the Cavendish subgroup (Israeli et al. 1991), but they have always been associated with fruit size and quality.

The objective of this study was to determine morphological differences between clone FHIA-21 and two lines developed by inducing mutations of FHIA-21.

Materials and methods

Research was conducted at the Instituto de Investigaciones en Viandas Tropicales (INIVIT) in Santo Domingo, Villa Clara, Cuba, from 1998 to 2005. The trials were sown in a brown soil with carbonate differentiation according to Hernández (1995). The plant material used was a clone of plantain FHIA-21 (AAAB) generated through in vitro culture. Crop management practices used were those recommended in the technical manual on the cultivation of plantain produced by the Cuban Ministry of Agriculture.

During field evaluation, out of a total population of 3333 plants, we detected two that had phenotypic traits different from those of the original cultivar (FHIA-21). The plants were identified as INIVIT-1 and INIVIT-2, and their subsequent populations, obtained through asexual reproduction, were evaluated under field conditions for two consecutive years with the purpose of determining their genetic stability.

Morphological characterization of INIVIT-1 and INIVIT-2 was carried out using Musa descriptors (INIBAP/IPGRI/CIRAD 1996). Characterization focused on 121 traits related to the plant, buds and male inflorescence, and to the flowers and fruit. Highly discriminatory descriptors that contribute most to genetic variability were emphasized and compared to those evaluated in the donor clone FHIA-21.

Results and discussion

During the period in which the lines were studied, their qualitative and quantitative phenotypic traits were stable, which suggests that the changes that had occurred were not influenced by the environment, but had been induced by in vitro culture.

INIVIT-1 has very drooping leaves compared to FHIA-21, which has drooping leaves, and INIVIT-2, which has a semi-erect leaf habit.

The pseudostem of INIVIT-1 has a homogeneous reddish-green hue compared to the donor clone FHIA-21 (medium green) and INIVIT-2 (greenish tint). The dorsal surface of the cigar leaf of INIVIT-1 has a purplish brown pigmentation while on FHIA-21 and INIVIT-2 it is green. The water suckers of INIVIT-1 and FHIA-21 have small or narrow dark brown spots, while those of INIVIT-2 have large purple spots.

The bracts are different in the two lines compared with the donor clone. The bracts of INIVIT-1 have a pointy end and are purple, while the ones of INIVIT-2 are purple and split at the end, and the ones of FHIA-21 are rounder at the end and are blackish purple.

The fruits of INIVIT-1 and FHIA-21 are of similar length, between 21 and 25 cm, while in INIVIT-2 the fingers are shorter (≤ 15 cm). The rachis of INIVIT-2 has male flowers and no persistent bracts. All these traits, and its lower reproductive potential, make INIVIT-2 different from the donor clone FHIA-21 and from INIVIT-1. In INIVIT-1, the colour of the unripe peel is medium green and the fingers are less
pointed than the ones of FHIA-21, while the peel of INIVIT-2 has a greenish hue. The pulp of FHIA-21 and INIVIT-1 is cream coloured, while INIVIT-2 has orange coloured pulp.

As for pollen fertility, we found that INIVIT-1 is similar in that respect to the donor clone FHIA-21, with a high percentage of fertile grains (91%), while INIVIT-2 has a very low number of pollen grains, none of which were found to be fertile in lab analyses. We recommend continuing to study these lines from cytogenetic, isoenzimatic and molecular perspectives. Their yield and agronomic performance in the field should also be evaluated for use in farm production.

Response of banana dwarf somaclonal variants to benzylaminopurine

K. Matsumoto, L. Styer Caldas and Y. Yamamoto

S
hoot-tip culture has commonly been used to micropropagate a wide range of Musa genotypes (Cronauer and Krikorian 1984, Vuylsteke 1998). Micropropagated plantlets grow faster, permit more synchronized harvesting and have higher yields than conventional planting material (Drew and Smith 1990, Robinson et al. 1993, Alvares and Caldas 2002). One of its disadvantages is the production of somaclonal variants. In Musa plants, the rate of somaclonal variation produced by shoot-tip culture varies between 0 and 25%, but can reach 70% in some plantain cultivars and 100% in extreme cases (review by Côte et al. 1998).

The intriguing thing is that about 80% of somaclonal variants are dwarf types (Reuveni and Israeli 1990). Thus, many studies have been undertaken to detect dwarf variants at early stages of development, using gibberellic acid (Damasco et al. 1996, Sandoval et al. 1999), isozyme profile polymorphism (Carvalho et al. 1998) and molecular markers (Damasc et al. 1998, Grajal-Martin et al. 1998). Some somaclonal variants originate from chimeric tissue in the explants (Crouch et al. 1998). High concentrations of growth regulators in the culture medium and prolonged subculturing also induce somaclonal variations (Reuveni and Israeli 1990, Shepherd et al. 1996, Rodrigues et al. 1998, Santos and Rodrigues 2004). However, they do not explain the very high frequency of dwarf variants. The high frequency may be caused, at least in part, by the unintended selection during micropropagation of dwarf variants. The objective of this study was to compare the in vitro multiplication rates of dwarf variants with those of true-to-type plants.

Materials and methods

The cultivars used in this study were ‘Nanicão’ and ‘Grand naine’. Both belong to the Cavendish subgroup (Musa AAA). In ‘Nanicão’, shoot tips were extracted from suckers and cultivated on Murashige Skoog (MS) medium supplemented with 30 g/L sucrose, 5 mg/L 6-benzylaminopurine (BA) and 1.5 or 2 g/L Phytagel. The multiple shoots were subcultured on the same medium at 30- to 45-day intervals for 2 years. The roots were then induced on MS medium with 0.5 mg/L α-naphthalene acetic acid (NAA).

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IPGRI/INIBAP/CIRAD. 1996. Banana (Musa spp.) Descriptors for banana (Musa spp.). International Plant Genetic Resources Institute, Rome, Italy; International Network for the Improvement of Banana and Plantain, Montpellier, France; and Centre de coopération internationale en recherche agronomique pour le développement, Montpellier, France.


More than 500 plantlets were acclimatized and transplanted to the field. Two years after transplantation, pseudostem height (from the base of the pseudostem to the base of the peduncle) was measured. Leaf overlap was also observed to identify dwarf variants. Of the 125 plants that fruited, 23 (18%) were true-to-type with regards to height, while 84 (67%) showed dwarfism and 18 (15%) had intermediate characteristics.

Thirteen of the true-to-type plants and 17 of the dwarf plants were randomly selected. Each parent plant was considered as a separate line during subsequent *in vitro* culture. Two suckers from each parent were re-established *in vitro*. The explanted shoot tips were cultivated on MS medium supplemented with 30 g/L sucrose, 2 g/L Phytagel and 3 mg/L or 10 mg/L BA. The cultures were maintained under a 16h:8h light/dark cycle at a light intensity of 42 \(\mu \text{mol m}^{-2} \text{s}^{-1}\) at 28 \(\pm\) 2°C and subcultured at monthly intervals using the same medium. The number of shoots was evaluated three months (two subcultures) after the initiation of culture.

The same process was also carried out with ‘Grand naine’. Five true-to-type plants and five dwarf plants were randomly selected from the two-year-old plantation and re-established *in vitro*. The culture conditions were the same as those used for ‘Nanicão’.

### Results and discussion

Although higher, the mean number of shoots produced by the dwarf lines of ‘Nanicão’ was not significantly different from the one produced by the true-to-type plants at both concentrations of BA (Table 1). Similarly, increasing the concentration of BA did not significantly increase shoot production in both dwarf and true-to-type plants.

In ‘Grande naine’, however, increasing the concentration of BA from 3 to 10 mg/L significantly increased the mean number of shoots produced by dwarf lines. Moreover, the dwarf lines grown on the medium with 10 mg/L BA also produced 40% more shoots than the true-to-type plants (Table 2). Reuveni and Israeli (1990) reported that the multiplication rate of dwarf plants did not significantly differ from that of the normal ones. They had used the cultivar ‘Williams’ and the Ma and Shii (1972) medium: 2 mg/L indoleacetic acid (IAA) with 5 mg/L kinetin or 200 mg/L tyrosine with 2 mg/L IAA and 5 mg/L BA.

Many commercial micropropagation laboratories now use only 3 to 5 mg/L BA or low concentrations of IAA and BA for their multiplication media (review by Matsumoto and Silva Neto 2003). The media used by Reuveni and Israeli (1990) showed the possibility of using medium to suppress dwarf variant multiplication.

If the somaclonal variants in our study had been chimeric mutants their production would have been reduced by subculturing. Roux et al. (2001) observed that cytochimeras induced by colchicine treatment were reduced from 100% to 36% after three subcultures, using a shoot-tip culture technique, and from 100% to 8%, using a multi-apexing technique. Instead, we observed that the proportion of dwarf variants increased with subculturing, reaching 67% of the micropropagated

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<table>
<thead>
<tr>
<th>BA concentration</th>
<th>Dwarf lines (Mean ±s. d.)</th>
<th>True-to-type lines (Mean ±s. d.)</th>
<th>Significance level (p) of two-sided t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 mgL</td>
<td>19.6 ± 4.7</td>
<td>16.1 ± 6.6</td>
<td>0.2108</td>
</tr>
<tr>
<td>10 mgL</td>
<td>23.5 ± 7.5</td>
<td>17.6 ± 1.7</td>
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<td>s. d.: standard deviation of two-sided t-test</td>
<td>0.1145</td>
<td>0.9996</td>
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<table>
<thead>
<tr>
<th>BA concentration</th>
<th>Dwarf lines (Mean ±s. d.)</th>
<th>True-to-type lines (Mean ±s. d.)</th>
<th>Significance level (p) of two-sided t-test</th>
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</thead>
<tbody>
<tr>
<td>3 mgL</td>
<td>14.1 ± 2.9</td>
<td>10.2 ± 6.2</td>
<td>0.2604</td>
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<td>10 mgL</td>
<td>19.9 ± 2.2</td>
<td>14.2 ± 2.6</td>
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<td>s. d.: standard deviation of two-sided t-test</td>
<td>0.0146</td>
<td>0.2417</td>
<td>-</td>
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</table>
plants after 2 years (about 20 subcultures). Rodrigues et al. (1998) reported an increase in the frequency of dwarf variants (evaluated as modified insertion of the leaves) after 7 to 11 subcultures and Santos and Rodrigues (2004) observed a similar effect in the Pacovan cultivar. Jambhale et al. (2001) also observed similar results after 10 to 14 subcultures. The origin of the dwarf variants is more likely caused by the in vitro conditions.

There is evidence that in vitro conditions will cause mitotic instability (Shepherd and dos Santos 1996). DNA methylation can also cause some somaclonal variation (James et al. 2004). These observations may explain low proportions of the somaclonal variants but not frequencies of more than 50%. Lane and Looney (1982) showed that a high level (10 μM) of BA in the culture medium selects for dwarf mutants in apple. We suggest that the high incidence of dwarf variants in banana micropropagation may be partly explained by their higher capacity for shoot production during micropropagation, so that they represent a relatively larger percentage of the total population as the number of subcultures increases.

References


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Response of cooking banana genotypes to fragmentation and incision during shoot tip culture

E.N. Adaoha Mbanaso, J. Crouch and F. Onofeghara

Induction of shoot multiplication during micropropagation is usually achieved by the inclusion of plant growth regulators, particularly cytokinins, in the culture medium. For plantain and cooking banana, relatively high concentrations of cytokinins are used (Vuylsteke 1989 a and b). Nevertheless, some workers (Cronauer and Krikorian 1984, Novak et al. 1990, Mbanaso et al. 2000) have attempted to manipulate Musa shoot tips to stimulate further increase in shoot production. The need to rapidly multiply propagules is particularly important during the dissemination of elite, exotic or improved genotypes. This study was carried out to assess the response of cooking banana genotypes, including plantain, to incision and fragmentation during shoot tip culture as a low-cost measure to increase and accelerate the build up of propagules during micropropagation.

Materials and methods

This study was carried out at the International Institute of Tropical Agriculture (IITA) Onne, Rivers State, Nigeria. Six genotypes, two plantain hybrids (TM3X 15108-6 and TMPX 548-9, AAB), a plantain cultivar ‘Obino I’ewai’ (AAB), two cooking banana hybrids (TMBX 612-74 and TMBX 5295-1, ABB), and the cooking banana cultivar ‘Cardaba’ (ABB), were used in the study. Shoot tips were excised from four-week-old in vitro plantlets and manipulated as follows:

1) Fragmented vertically into 4 equal portions
2) Fragmented vertically into 2 equal portions
3) A vertical incision at the apical region of the shoot tip
4) A vertical incisions at the basal region of the shoot tip
5) Control; unfragmented and no incision.

The plants were not weighed since the comparisons were based on type rather than size of explants. The incisions were sufficiently deep for the tip of the blade to slightly appear on the opposite side of the shoot tip, without splitting it. Explants were cultured on a modified Murashige and Skoog (1962) medium adopted for Musa culture at IITA (Vuylsteke 1989 b). Gelrite (Sigma Chemical Company, St Louis, USA) at 2 g/L was used to solidify the medium. The pH was adjusted to 5.8 before autoclaving at 121°C and 1.05 kg/cm² for 15 minutes. Explants were seeded individually in test tubes (25 mm x 150 mm) containing 20 ml culture medium, capped with autoclavable closures and arranged on racks in a completely randomized design.

Each treatment was made up of 24 explants per genotype and each experiment was repeated twice. Cultures were incubated at 27±2°C, under a 14 h photoperiod delivered by white fluorescent tubes producing irradiance in the range of 30 to 40 Mmol m⁻² s⁻¹. After 4 weeks in culture, survival of explants, number of shoots produced per explant seeded and height of shoots were scored. This work was repeated at least twice. Computer programmes for the statistical analysis of data generated during the study were constructed using PC-SAS (SAS Institute 1992). The General Linear Model procedure of SAS was used for the analysis of variance (Crompton 1994). The least significant difference (LSD) served to separate the means.

Results

Fragmentation did not affect the survival of the plantain hybrid TM3X 15108–6 (Table 1) and the cooking banana hybrid TMBX 5295–1 (Table 2). The survival of the fragmented explants of the plantain hybrid TMPX 548-9 and cultivar ‘Obino I’ewai’ was significantly reduced. Of the other two genotypes, only the explants of the cooking banana hybrid TMBX 612–74 fragmented into four parts had a lower survival than the control explants.

Incised explants produced significantly more shoots per explant than control explants in the plantain hybrids only (Tables 1 and 2). They also produced more shoots per explant than the fragmented explants except for the explants fragmented into two of the plantain...
In this study, fragmentation resulted from wounded tissue (Vuylsteke 1989b). Blackening is believed to interfere with nutrient uptake, leading to inhibition of growth and consequently death (Vuylsteke 1989b). In this study, fragmentation resulted in explants with more exposed surfaces that produced more exudates, which oxidized to cause browning, thus explaining the higher mortality among them. The hybrids TM3X 15108-6 and TMBX 5295-1 survived better than the other genotypes to fragmentation. Much variability does exist among Musa cultivars in their response to blackening in culture (Hirimburegama and Gamega 1997). The observation that incisions increased shoot production is in agreement with Vuylsteke (1989). Although this procedure is not essential for multiple shoot production in the presence of cytokinin, it improves the process and does not depend on polarity. The plantain genotypes tended to respond better than the cooking banana genotypes. This could reflect genotypic differences.

Fragmentation can be used to inexpensively increase the number of shoots produced in Musa cultivars at the multiplication stage, particularly when initial numbers are small, as on the receipt of germplasm. For the production of shoots for rooting, whole explants, with or without incisions, would serve better. The diversity of responses to culturing among Musa genotypes is noteworthy and may be useful for breeding programs.

**Discussion**

Findings from the present study indicate that fragmentation of the shoot tip impaired the ability of the explants to survive in culture. Mortality of Musa shoot tip explants in culture has been attributed in part to blackening caused by the oxidation of phenolic compounds which exude from wounded tissue (Vuylsteke 1989b). Blackening is believed to interfere with nutrient uptake, leading to inhibition of growth and consequently death (Vuylsteke 1989b). In this study, fragmentation resulted

<table>
<thead>
<tr>
<th>Treatments</th>
<th>TM3X 15108-6</th>
<th>TMBPX 548-9</th>
<th>Obino 'Iewai</th>
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<tr>
<td>Survival (%)</td>
<td>Number of shoots per explant</td>
<td>Number of shoots per explant</td>
<td>Number of shoots per explant</td>
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<tr>
<td>TM3X 15108-6</td>
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<td></td>
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<tr>
<td>Fragmented in 4 parts</td>
<td>97.9 a</td>
<td>4.4 b</td>
<td>1.6 b</td>
</tr>
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<td>100.0 a</td>
<td>4.7 b</td>
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</tr>
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<td>Incisions at the apex</td>
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<td>6.3 a</td>
<td>1.7 a</td>
</tr>
<tr>
<td>Incisions at the base</td>
<td>97.9 a</td>
<td>7.2 a</td>
<td>1.5 b</td>
</tr>
<tr>
<td>Control</td>
<td>97.9 a</td>
<td>5.0 b</td>
<td>2.1 a</td>
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</tbody>
</table>

Means in a column followed by different letters are significantly different at p=0.05 according to the Least significant difference test.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>TMBX 612-74</th>
<th>TMBX 5295-1</th>
<th>Cardaba</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival (%)</td>
<td>Number of shoots per explant</td>
<td>Number of shoots per explant</td>
<td>Number of shoots per explant</td>
</tr>
<tr>
<td>TMBX 612-74</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fragmented in 4 parts</td>
<td>81.3 b</td>
<td>3.4 b</td>
<td>0.9 c</td>
</tr>
<tr>
<td>Fragmented in 2 parts</td>
<td>93.6 a</td>
<td>4.9 a</td>
<td>1.4 bc</td>
</tr>
<tr>
<td>Incisions at the apex</td>
<td>100.0 a</td>
<td>5.9 a</td>
<td>1.3 bc</td>
</tr>
<tr>
<td>Incisions at the base</td>
<td>97.9 a</td>
<td>5.0 a</td>
<td>2.0 a</td>
</tr>
<tr>
<td>Control</td>
<td>97.9 a</td>
<td>5.8 a</td>
<td>1.5 b</td>
</tr>
</tbody>
</table>

Means in a column followed by different letters are significantly different at p=0.05 according to the Least significant difference test.
Metabolic importance of starch in the acclimation of plantain ‘CEMSA ¾’ (AAB) plants


Plants that have been micropropagated using in vitro culture must undergo an acclimation process, during which the quantity and quality of the reserves the plants have managed to store play a vital role. Survival during acclimation depends on the plants being able to make the transition from heterotrophic or mixotrophic (a combination of autotrophic and heterotrophic) conditions to autotophism. Plants must adapt to external environmental conditions that are different from those of in vitro culture, e.g. light intensity is higher than the intensity they experienced during in vitro culture, and relative humidity is lower. These differences can cause plants to dry up and die. The presence of sucrose in the culture media is also important, given that under external environmental conditions plants only have water and mineral salts present in the soil. During acclimation, the plants’ metabolism and leaf shape change to adapt to external conditions. This happens at the expense of reserve substances such as starch.

Starch metabolism provides useful metabolic elements that can be used to describe and analyse leaf development, for starch is the final product of the physiological activity of the various crops that use it as an energy reserve (Bello-Pérez et al. 2002). For this reason, we decided to track starch metabolism through the starch-synthesizing enzyme ADP-glucopyrophosphorylase. Knowledge of the key moments and mode of starch mobilization makes it possible to identify the acclimation stage that requires more care and on which the plant’s survival depends.

Materials and methods

Plants of plantain CEMSA ¾ (AAB) were produced using in vitro culture in Temporary Immersion Bioreactors (TIBs). The TIBs consisted of two transparent 250 ml Nalgene beakers. One hundred milliliters of culture medium (10 ml/explants) were added to the beakers. One hundred milliliters of culture medium (10 ml/explants) were added (Escalona et al. 1999). The plants spent 28 days in the multiplication phase and 21 days in the growth phase.

Plants ready for acclimation must have a pseudostem thickness greater than 0.3 cm, at least two expanded leaves, and a height greater than 2.5 cm. The plants that met those requirements were transferred to a sterile substrate containing 1:1 sugar cane-based compost and red soil placed in 144-well trays (52.5 cm x 29.5 cm x 4 cm). The plants to be acclimated were placed in a growth chamber and kept at 28±1°C average temperature, 80-90% relative humidity, an atmospheric concentration of CO₂ and an alternating light-darkness regime.
All quality parameters of the plants were measured every seven days during acclimation. Fresh and dry mass were determined (72 h at 70°C) after measuring the gaseous interchange.

Completely expanded leaves were used to determine the plants’ maximum photosynthetic capacity, four or five hours after the onset of the alternating light-darkness regime. Maximum photosynthetic capacity and transpiration were measured using a CIRAS-2 (Portable Photosynthesis System, Europe, PP Systems, UK) hooked up to a universal container (PLC6). The container was covered with the youngest fully expanded leaf (2.5 cm²). Carbon dioxide concentration and relative humidity reached 375 µmol/mol and 80%, respectively, under controlled light conditions (600 µmol m⁻² s⁻¹).

To determine starch concentration, 1.0 g of fresh mass was ground up in a mortar with liquid nitrogen. The sugars were extracted in 5.0 ml of 80% ethanol. Starch was hydrolyzed according to the protocol described by Thomas et al. (1983). Starch was quantified based on the previously constructed curve pattern for potato starch.

To extract and measure the enzymatic activity of adenosine diphosphate-glucopyrophosphorylase (ADP-GPPase), 250 mg of leaf segments were placed directly in liquid nitrogen and ground up in a mortar. Enzymes were extracted following the method described by Geigenberg and Stitt (1991). Enzymatic activity was determined by adding 100 µl of vegetable extract to 50 mmol/L of buffer solution. The glucose-1P formed was quantified according to the procedure described by Smith (1990).

Results and discussion

All of the plants’ morphological quality indicators reached maximum development on day 35 of the acclimation process (Table 1). Indicators such as the number of leaves remained the same after the first seven days, and the leaves grew both in length and in width. Other indicators such as rhizome diameter reached their maximum values at 35 days. Fresh weight reached its peak in the last week of acclimation, while dry weight stayed the same after day 21.

The number of roots remained the same throughout the acclimation process. This shows that the roots developed during the in vitro phase in a TIB last through the entire acclimation process, and the continuous increase in root length confirms they were functional. In a TIB, the gaseous environment is frequently renewed to oxygenize plant roots, in contrast to what happens to roots grown on a semi-solid culture medium (Preece and Sutter 1991).

The survival rate was 96.5% between the 14th and 35th days of acclimation. The plants survived thanks to the quality of the morphological indicators such as plant height, fresh and dry weight, and number of functional roots. For plants to adapt to environmental conditions early in the acclimation process, they need to have stored carbohydrate reserves during the growth stage, also, their physiological development should have reached a level where their photosynthesis and transpiration rates are similar to those of adult plants.

When the plants were transferred to the growth chamber, they quickly adapted to autotrophic growing conditions, which was evidenced by the marked change in their photosynthetic activity, which reached between 9.2 and 11.5 µmol CO₂ m⁻² s⁻¹ (Table 2). These values are similar to those reported for adult plantains under field conditions (Cayón 2001).

Photosynthetic activity decreased after 35 days, perhaps because the plants reached their maximum development at 35 days or that the amount of nutrients supplied by the substrate was insufficient at 42 days. At this stage, plant growth may be limited by the

<table>
<thead>
<tr>
<th>Days</th>
<th>Plant height (cm)</th>
<th>Rhizome diameter (cm)</th>
<th>Number of leaves</th>
<th>Leaf length (cm)</th>
<th>Leaf width (cm)</th>
<th>Number of roots</th>
<th>Root length (cm)</th>
<th>Fresh weight (g/cm²)</th>
<th>Dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.0 d</td>
<td>0.51 c</td>
<td>4.5 b</td>
<td>2.9 d</td>
<td>1.1 d</td>
<td>4.5 a</td>
<td>3.3 d</td>
<td>2.9 c</td>
<td>0.14 b</td>
</tr>
<tr>
<td>7</td>
<td>10.0 c</td>
<td>0.49 c</td>
<td>6.0 a</td>
<td>5.3 c</td>
<td>1.9 c</td>
<td>6.2 a</td>
<td>4.1 d</td>
<td>2.8 c</td>
<td>0.15 b</td>
</tr>
<tr>
<td>14</td>
<td>12.4 b</td>
<td>0.56 bc</td>
<td>7.0 a</td>
<td>6.8 b</td>
<td>2.6 b</td>
<td>5.8 a</td>
<td>9.0 c</td>
<td>3.6 b</td>
<td>0.14 b</td>
</tr>
<tr>
<td>21</td>
<td>14.5 ab</td>
<td>0.62 bc</td>
<td>7.2 a</td>
<td>8.7 a</td>
<td>3.3 a</td>
<td>5.6 a</td>
<td>13.2 b</td>
<td>4.4 b</td>
<td>0.17 b</td>
</tr>
<tr>
<td>28</td>
<td>14.5 ab</td>
<td>0.72 abc</td>
<td>7.2 a</td>
<td>9.0 a</td>
<td>3.3 a</td>
<td>6.2 a</td>
<td>14.2 b</td>
<td>4.6 b</td>
<td>0.27 a</td>
</tr>
<tr>
<td>35</td>
<td>16.1 a</td>
<td>0.78 a</td>
<td>6.2 a</td>
<td>10.2 a</td>
<td>3.6 a</td>
<td>4.8 a</td>
<td>16.6 a</td>
<td>5.7 a</td>
<td>0.28 a</td>
</tr>
<tr>
<td>63</td>
<td>16.5 a</td>
<td>0.82 a</td>
<td>6.4 a</td>
<td>10.3 a</td>
<td>3.8 a</td>
<td>5.0 a</td>
<td>19.0 a</td>
<td>6.4 a</td>
<td>0.29 a</td>
</tr>
</tbody>
</table>

Means followed by different letters in the same column indicate significant differences with a 5% degree of confidence using Tukey’s test.
Starch concentrations in the rhizome decreased to levels closer to the ones in the leaf and remained that way until the 35th day of the acclimation process. Perhaps in the late developmental stages under field conditions, the plantain plants will once again accumulate starch in the rhizome to provide enough energy for the development of the suckers and reproductive structures.

The ADP-GPPase enzy-matic activities during acclimation are presented in Table 4. These activities are greater in the rhizome at the end of the in vitro phase, and are related to the rhizome’s capacity for storing large amounts of starch. The enzymatic capacity of the rhizome and leaves is reversed later on. Starting at 21 days, the leaves’ progressive development increases the ADP-GPPase activity, along with studies on photosynthesis (Table 2) revealed that in the first seven days of exposition to external conditions, there was a large increase in transpiration caused by the transfer to an environment with lower relative humidity and higher light intensity. After that time, the plants slowly reduced their transpiration rate, a basic process that shows their adaptability. Precisely one week after the start of acclimation, there was an increase in the various morphological indicators (plant height and fresh mass, among others) due to water retention (Table 1).

In vitro culture in a TIB facilitates the renewal of the environment, which allows the stomata to reach a certain degree of functional capacity, thus avoiding water loss. Plants lose water in two ways, through the stomata and through the leaf cuticle, which involves the entire leaf surface (Preece and Sutter 1991). In a TIB, the plants are bathed every three hours, which may favor leaf cuticle development and reduces water loss through the cuticle. Higher photosynthesis/transpiration values are associated with greater functional capacity of the stomata, which allow CO₂ to enter and little water to be lost through the leaves. The highest photosynthesis/transpiration ratio (5.6) was reached at 35 days.

Results of starch concentration tests conducted early in the acclimation process show that plants accumulate more starch in the rhizome than in the leaves (Table 3). In the first seven days of acclimation, a reduction in starch concentration both in the leaves and rhizome was observed. The reduction was more pronounced in the rhizome because it is genetically programmed to store energy-rich compounds; this gives it a greater capacity for starch mobilization. The fact that the rhizome has no chlorophyll and is underground in the field confirms that it is a storage organ. Starch metabolism at this stage provides the plants with the energy they need to develop in the first two weeks, when they are still unable to obtain enough energy through photosynthesis, and when they need to reprogram the entire leaf cell metabolism.

### Table 3. Mean starch concentration in the leaves and rhizome of plantain CEMSA ¾ (AAB) plants during 35 days of acclimation (n=9)

<table>
<thead>
<tr>
<th>Days</th>
<th>Starch concentration (mg/g MF)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf</td>
</tr>
<tr>
<td>0</td>
<td>48.96 b</td>
</tr>
<tr>
<td>7</td>
<td>24.91 c</td>
</tr>
<tr>
<td>14</td>
<td>25.53 c</td>
</tr>
<tr>
<td>35</td>
<td>39.85 b</td>
</tr>
</tbody>
</table>

Means followed by different letters in the same column indicate significant differences with a 5% degree of confidence using the Student-Newman-Keuls test.

### Table 4. Enzymatic activity of ADP-glucopyrophosphorylase (ADP-GPPase) in the leaves and rhizome of plantain CEMSA ¾ (AAB) plants during acclimation (n=9)

<table>
<thead>
<tr>
<th>Days</th>
<th>Enzymatic activity of ADP-GPPase (U/g MF)* (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf</td>
</tr>
<tr>
<td>0</td>
<td>0.68 b</td>
</tr>
<tr>
<td>7</td>
<td>0.70 b</td>
</tr>
<tr>
<td>14</td>
<td>0.70 b</td>
</tr>
<tr>
<td>35</td>
<td>0.88 a</td>
</tr>
</tbody>
</table>

*1 U = 1 µmol of substrate transformed per hour. Means followed by different letters in the same column indicate significant differences with a 5% degree of confidence using the Student-Newman-Keuls test.
with their photosynthetic activity. In contrast to what happens under in vitro conditions, once plants have adapted to the external environment, the leaves become the main starch-synthesizing organ, although what they actually synthesize is ADP-glucose, a precursor, which is then transferred to the rhizome for starch synthesis and storage.

The acclimatisation process of plantain plants cultured in a TIB was completed in 35 days, at which time it became necessary to transplant. Also at 35 days, the morphological indicators reached their maximum development after which photosynthetic activity went down. Functional roots formed during the in vitro stage favored the plants’ acclimatization process. The first seven days of acclimation are crucial for plant adaptation, and leaf metabolic development is essential for photosynthesis, transpiration, and starch metabolism. In the first seven days, the plants survive thanks to the energy that was stored in the rhizome as starch during the in vitro stage.

References


Highlights from a survey

I. Van den Bergh

In 1989, INIBAP set up the International Musa Testing Programme (IMTP) to evaluate—using shared methodologies to ensure comparison of the results across sites—the banana hybrids produced by breeders. Following the first phase of this collaborative effort, in which six countries participated, two dessert hybrids, FHIA-01 and FHIA-02, and one cooking banana hybrid, FHIA-03, were recommended for release. Since then, these three hybrids have been distributed to more than 50 countries. The second phase, which started in 1996, singled out FHIA-23 and SH-3436-9 as the most tolerant to black leaf streak disease.

At present, 25 countries are participating in the third phase and for the first time two private companies are also participating in the trials in Asia. Before embarking on the data analysis, INIBAP did a survey amongst various groups of stakeholders to evaluate the programme and its usefulness.

Profile of the respondents

Exactly 100 people (or 38% of the contacted people) completed the questionnaire. The highest proportion of respondents works in Asia and the Pacific (44%), followed by Africa (32%) and Latin America and the Caribbean (18%). Europe and North America accounted for 10 and 2% of the responses, respectively. It should be noted that the survey was available only in English, a factor that may have influenced the response rate from certain regions.

The majority of the respondents are specialized either in plant protection (including pathology, nematology and entomology), or in Musa genetic improvement (including breeding, biotechnology, molecular biology and genomics) (Figure 1).
Representatives from 16 of the National Agricultural Research Systems (NARS) participating in the INIBAP regional networks shared their views. Just over 60% of the respondents are a member of ProMusa or one of its working groups, and 55% participated in one or more of the previous phases of the IMTP.

**General appraisal**

Seventeen years after the start of the programme, the IMTP is still highly commended by the stakeholders. 91% of the respondents feel that the IMTP trials are useful. Among the reasons to participate in the IMTP, many cited the benefits stemming from participating in international research activities and sharing the results within the *Musa* research community. Some respondents said they agreed to take part in the IMTP because they are involved in the development of the materials or in the collection of wild banana species, or because it is the mandate of their institute or research group, and they have the capacity to carry out IMTP trials.

More than 70% of the respondents who were not involved in any of the previous trials would have wanted to participate. Seven of them said they didn’t because it is not within their field of expertise or within the mandate of their institute. A lack of facilities and funding were also mentioned, as well as the existence of severe restrictions on the introduction in their country of banana germplasm. Phase II was also seen as quite cumbersome and intractable by some.

The major constraint encountered during the previous IMTP trials was the lack of funds, followed by time constraints (Table 1). Both a shortage of fields with good infestation levels of pests and diseases and a lack of trained staff were also seen as problems, as was the poor acceptability of the new hybrids by growers and consumers.

Plant pathologists agree with the principle of testing varieties under different environmental conditions, pathogen populations and production systems to get more reliable information about the overall performance of a cultivar. According to them, the identification by NARS, and indirectly by farmers, of useful cultivars suitable for local conditions was another reason to support the programme. Breeders, on the other hand, mainly stressed its value for exchanging germplasm and accessing new materials.

Most respondents believe that breeders are the most interested in the results of the IMTP, followed by farmers and plant pathologists. Agronomists and other banana researchers are two other important target groups. Many respondents felt that information collated from the IMTP trials is essential for managing pests and diseases, as using resistant varieties is considered to be the best long-term solution to manage many of the major pests and diseases.

### Table 1. Major constraints encountered by respondents who had participated in one of the IMTP trials.

<table>
<thead>
<tr>
<th>Constraint</th>
<th>% of respondents</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insufficient funds</td>
<td>45</td>
<td>The time needed to produce the required quantities of plantlets often results in a shortage of planting material at the start to sufficient quantities of an experiment. This is aggravated by the requirement of using resistant or susceptible checks from the INIBAP Transit Centre.</td>
</tr>
<tr>
<td>Lack of time</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Slow multiplication of germplasm</td>
<td>17</td>
<td>The trial design is seen as too complicated for the experience level of staff, a situation that could affect the reliability of the data. In that respect, some respondents also found the scientific quality control by INIBAP insufficient.</td>
</tr>
<tr>
<td>Lack of fields with good infestation levels of pests and diseases</td>
<td>17</td>
<td>More and better follow-up as well as improved communication between IMTP partners would ensure data of better quality and stimulate interaction and participation. Constraints and shortcomings could be resolved earlier. A faster, more detailed data analysis using modern tools as well as a better feedback and distribution of results was also recommended.</td>
</tr>
<tr>
<td>Lack of trained staff</td>
<td>14</td>
<td>Despite their high yield, many new hybrids are poorly accepted by growers and consumers because of their taste, lower cooking qualities, taller plant height and longer crop cycle.</td>
</tr>
<tr>
<td>Poor acceptability of hybrids</td>
<td>12</td>
<td>A lack of interest in banana, and especially <em>Musa</em> improvement, by national governments is sometimes a problem. Collaboration with other institutions, such as universities, may offer a solution to this constraint.</td>
</tr>
<tr>
<td>Abiotic stresses like typhoon and chills</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Lack of interest by government</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>
Information products

INFOMUSA was the single most important source from which people learned about the results of the previous phases of the IMTP (60% of respondents), followed by the 2000 book edited by G. Orjeda (27%) and the IMTP CD-Rom (22%). Other sources include the Phase I report by D. Jones, the abstract book of the 2004 International Conference on Banana and Plantain in Malaysia, technical and regional networks meetings, individual trial reports and discussions with banana and INIBAP scientists. One-fifth of the respondents reported not to have received any information about the results of the IMTP trials.

71% and 63% of the respondents found the CD-Rom and book very and somewhat useful and good instruments for disseminating knowledge about new varieties, their resistance to pests and diseases and their performance across sites. The CD-Rom was found more practical because it contains more information and can be copied. Some people, however, said that the search function is not very user-friendly and that they were not able to read the CD-Rom. Among the other weaknesses mentioned, the descriptive statistics were not deemed very useful; the results are not presented in a comprehensive format; the write-up is sometimes vague and unconvincing; more information could have been extracted by doing a more in-depth analysis and the information was not well disseminated.

All respondents agreed that scientific publications are useful to present the results of the IMTP, and almost 90% of the respondents think a catalogue would be useful, preferably with more agronomic information than the one currently found in Musologue (Table 2). A website got an intermediate rating, while online publications and an online database got the weakest support.

Support for a fourth phase

There was a broad consensus that evaluating recently developed germplasm for its reaction against leaf spot diseases, Fusarium wilt and nematodes should be a continuous process and part of all genetic improvement efforts. Only four people said they would not participate, but only because they do not have the facilities or the expertise to participate in such a trial.

86% of the respondents would participate in a fourth phase but most of them said they would only if certain conditions are met. Almost one-fourth said they would participate only if they were funded (by their national government, the industry or INIBAP) and were provided additional backstopping. More involvement in what happens after the data are sent in and receiving due credit were also mentioned as important conditions for participating. Some respondents noted that the decision to participate was not up to them.

A new phase of the IMTP could also address the limitations and shortcomings of the previous phases. It was also felt that it would offer more opportunities to collaborate and share knowledge towards a common goal.

Suggestions for the future

New improved materials such as hybrids and somaclonal mutants are still viewed as the most important material for evaluation by 74% of the respondents, but more than half of the respondents would also like to include popular cultivars from various countries.

Three of the pests and diseases against which material is currently tested, namely Fusarium wilt, black leaf streak and nematodes, are considered central to future IMTP trials (Table 3). Eumusa leaf spot was considered the least important biotic factor listed.

The importance of the various pests and diseases varied with the region. In Latin America and the Caribbean, there was a very strong consensus that priority should be
given to evaluating germplasm against black leaf streak (100%), Fusarium wilt (92%) and nematodes (80%). In Asia, Fusarium wilt was ranked first (97%), followed by black leaf streak (78%). Bacterial wilt was considered important by 60% of the respondents. In Eastern and Southern Africa, high priority is given to Fusarium wilt (100%), bacterial wilt (89%), nematodes (79%), black leaf streak (75%) and weevils (75%). Respondents from West and Central Africa ranked nematodes first (100%), followed by black leaf streak (78%) and BBTV (60%).

Other factors that respondents felt should be included in future trials were consumer acceptability, agronomic performance and fruit quality (Table 4).

Nearly four-fifth of the respondents want to keep the two levels of evaluation: the performance and in-depth trials. Many respondents recommended more visits from INIBAP scientists to ensure the quality of the experiments. Several respondents also provided other suggestions on how to improve the programme (Table 5).

Only four people indicated that they have sufficient knowledge and don’t need any additional training to carry out an IMTP trial. Training needs are highest for diagnosis of pests and diseases and statistical analysis. Several respondents indicated that the technical guidelines are not sufficient and that all the staff participating in a trial need to be trained in the standard implementation of evaluation trials, in order to produce data that are comparable between sites. Human development and capacity building should be an integral part of the IMTP.

Some respondents said they would like to see more information on the interactions between genes and the environment to increase our knowledge on the stability of certain traits and help fine tune release strategies.

With regards to the presentation of the data, it was felt that graphical presentation of the results should be improved. There was a call for more publications in French and Spanish, and also for hard copies in colour for people without an easy access to computers. Products should be distributed

<table>
<thead>
<tr>
<th>Table 4. Percentage of respondents rating the usefulness of evaluating various characteristics in a new phase of IMTP.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Very important</strong></td>
</tr>
<tr>
<td>Consumer acceptability</td>
</tr>
<tr>
<td>Agronomic performance</td>
</tr>
<tr>
<td>Fruit quality</td>
</tr>
<tr>
<td>Potential for processing</td>
</tr>
</tbody>
</table>

Table 5. Suggestions to improve the programme.

- Involve biometricians in the design of trials
- Better standardization of procedures (e.g., for diagnosis and evaluation against pests and diseases)
- Trials should be carried out in areas infected with the pest or disease in question (and preferably not adjacent to plantations)
- Procedures to standardize the inoculation method for soil pests and diseases should be sought
- Participants should always use the same set of reference varieties but be allowed some freedom in the choice of other varieties
- Multilocational trials within a country
- Standard cultural practices to more accurately assess the real performance of the cultivars, not only under local agro-ecological conditions, but also under local farmers’ practices
- Involve NGOs and extension workers in evaluating agronomic performance
- Economic analysis
- Sufficient technical support and guidance
- More information on the different varieties should be provided at the onset
- Field diagnosis of pests and diseases should be confirmed in the laboratory
- Reliable disease diagnostic methods and tools are needed
- Early-screening experiments in the greenhouse (faster and cheaper than field tests) and methods of early selection using biotechnological tools
- Procedures for data collection should be well explained to the staff collecting the data
- Data should be collected regularly
- More detailed ecological data should be collected at all sites
- Well-designed and easy-to-use data collection sheets should be distributed to all partners
- Participants should be encouraged to use a log sheet to document the entire trial
- Involve biometricians in the analysis of the data
- Modern methods and appropriate software for data analysis
- More attention to analysis of gene-environment interactions
on a broader scale, e.g. through the regional information networks and/or a quarterly newsletter.

Other suggestions were aimed at improving communication and networking, such as discussion groups and an IMTP mailing list, as well as workshops and meetings. Many respondents recommended an annual global meeting, or regional ones, to help highlight difficulties and discuss with experts possible solutions.

Nearly three-quarters of the respondents said they were willing to share germplasm, but 40% of them specified that it could only be done under certain conditions, such as respecting national rules or getting approval from the management of the participating companies. A Material Transfer Agreement has to be in place. Sharing data and germplasm is conditional on everybody doing it and is an important factor in determining one’s willingness to share. Quarantine issues should also be considered.

**Acknowledgements**

We would like to thank all those who responded to the survey and assure them that their feedback will be taken into consideration when reviewing the IMTP.

For more information, contact Inge Van den Bergh at i.vandenbergh@cgiar.org

*Inge Van den Bergh works for INIBAP in Montpellier, France.*

**A staple food with nutritious appeal**

_C. Lusty, E. Akyeampong, M.W. Davey, G. Ngoh Newilah and R. Markham_

Some of the earliest archaeological evidence of organized agriculture in humid tropical Africa is found in central Cameroon (Mbida et al. 2000). It suggests that farmers in this part of the world have been cultivating Musa for more than 2000 years, actively selecting varieties and generating the high levels of plantain diversity Cameroonians enjoy today. In the process, these early farmers created varieties that are now sought for their nutritional qualities.

Recently, Lois Englberger’s work (Englberger 2003, Englberger et al. 2003) has highlighted the importance of orange-fleshed Musa as a source of provitamin A carotenoids (pVACs), plant-derived compounds that are converted to vitamin A in the human body. Vitamin A has a role in vision, as well as immunological, reproductive and embryo development functions. A deficiency of vitamin A in the diet represents one of the key challenges affecting the developing world. Up to half a million children are estimated to go blind every year from vitamin A deficiency and more than 50% of all deaths in any one year are associated with malnutrition (WHO 2003). These death rates would be higher if it wasn’t for the costly and regular interventions by NGOs and governments distributing vitamin and mineral supplements.

In 2004, a number of CGIAR centres formed an alliance, coordinated by the International Food Policy Research Institute and the Centro Internacional de Agricultura Tropical, under the name HarvestPlus Challenge Programme. The concept behind HarvestPlus is that micronutrients can be conveyed to vulnerable populations more cheaply and effectively through biofortified staple food crops. The programme places a strong emphasis on increasing productivity and nutrient density through crop improvement. The first phase of the programme focussed on evaluating the genetic variability of maize, wheat, rice, cassava, sweet potato and beans for three key micronutrients, iron, zinc and pVACs.

In a second phase, additional crops are being investigated and both the International Institute for Tropical Agriculture and the International Network for the Improvement of Banana and Plantain (INIBAP) of Bioversity International have been commissioned to carry out research on Musa. INIBAP’s work has involved bringing together a group of collaborators, the Centre Africain de Recherches sur les Bananiers et Plantains (CARBAP) in Cameroon, the Crop Research

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**Focus on nutrition**

_C. Lusty, E. Akyeampong, M.W. Davey, G. Ngoh Newilah and R. Markham_

Some of the earliest archaeological evidence of organized agriculture in humid tropical Africa is found in central Cameroon (Mbida et al. 2000). It suggests that farmers in this part of the world have been cultivating Musa for more than 2000 years, actively selecting varieties and generating the high levels of plantain diversity Cameroonians enjoy today. In the process, these early farmers created varieties that are now sought for their nutritional qualities.

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Institute and Food Research Institute in Ghana and the Katholieke Universiteit Leuven (KULeuven) in Belgium, to evaluate, among other things, the plantain cultivars that are held in the CARBAP field collection. CARBAP manages one of the largest collections of Musa, including a broad representation of cultivars from traditional farming systems in sub-Saharan Africa and the Pacific. This paper provides a review of the issues surrounding the study of pVACs and the activities of the INIBAP-coordinated HarvestPlus project.

What are carotenoids?

There are some 600 types of carotenoids known, of which approximately 50 play a role in the human diet (Rodriguez-Amaya 1997). Beta-carotene has the highest level of vitamin A activity, hence the importance of determining which carotenoids are present when evaluating the nutritional value of foods. Depending on the method used, analyses of carotenoids may supply values for:

- total carotenoids (all carotenoids including those that have no vitamin A activity),
- pVACs (carotenoids that have vitamin A activity),
- beta-carotene equivalents (provitamin A carotenoids converted into equivalent beta-carotene units)
- individual carotenoids (pVACs plus lycopene, lutein, etc).

Table 1 shows the methods used to quantify carotenoid levels in the HarvestPlus project.

Major constraints affect the interpretation and presentation of carotenoid analyses:

- Carotenoid content is highly variable within a plant and between plants and varieties. It also varies with fruit ripeness. This presents a substantial sampling challenge. The most appropriate sampling time and methods have to be established for comparative work.
- Carotenoids oxidize easily. Exposure to light, air and physical damage affect the rate of carotenoid loss once the sample is removed from the plant. Again this presents a challenge in terms of storing and transporting samples.
- Methods vary in their accuracy and precision. Results are often based on different analytical protocols, and are sometimes published without reference to what has been measured (total carotenoids or beta-carotene, fresh or dry weight, etc), and which methodologies were used. Processed materials may be directly compared with raw. Consequently, little standardized information exists to compare different foods or crops.

Once a value for beta-carotene equivalents has been determined, the nutritional value of the food (consumed in the form in which it was analysed) can be estimated using conversion factors for the absorption and metabolism of carotenoids in the body. The UN Food and Agriculture Organization uses a 1:6 ratio of Retinol Equivalents (RE) to beta-carotene and a 1:12 ratio for the other provitamin A carotenoids, based on the estimated absorption of 30% of the beta-carotene. The US Institute of Medicine more recently advised a 1:12 ratio of Retinol Activity Equivalents (RAE) to beta-carotene equivalents—the conversion rate used by HarvestPlus.

Table 1. Methods used in the INIBAP-coordinated HarvestPlus project for quantifying the carotenoid content in Musa fruit.

<table>
<thead>
<tr>
<th>Method</th>
<th>Tools</th>
<th>Type of results</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour assessment</td>
<td>Colour fans, charts and colorimeters</td>
<td>Ranking by colour as a proxy for total carotenoids</td>
<td>In Musa, there is a correlation between colour and carotenoid content. This method is cheap and quick for ranking varieties of the same species according to potential carotenoid content.</td>
</tr>
<tr>
<td>Spectrophotometry</td>
<td>Spectrophotometer</td>
<td>Total carotenoids</td>
<td>Effective method of quantifying total carotenoids. However, it is not possible to distinguish the range of individual carotenoids.</td>
</tr>
<tr>
<td>High performance liquid chromatography (HPLC)</td>
<td>HPLC system and diode array detector</td>
<td>Total provitamin A carotenoids, beta-carotene equivalents, individual carotenoids, cis and trans isomers.</td>
<td>Costly technique but the preferred means of quantifying specific carotenoids (e.g. beta and alpha-carotenes, lutein, etc.) and their geometric isomers.</td>
</tr>
</tbody>
</table>
Screening plantain

Plantain samples were flown fresh from the field to the laboratory in Leuven, where they were immediately prepared and frozen for later analysis using High Performance Liquid Chromatography (HPLC) and spectrophotometric methods. A standardized protocol for the HPLC screening of large numbers of Musa samples developed by Davey et al. (in press), resulted in increased throughput and reduced analysis time and costs.

Preliminary results suggest that the orange-fleshed plantain cultivars, which are locally popular in Cameroon, are significant sources of provitamin A carotenoids, although none so far is as rich as the Fe’i bananas studied in Micronesia (Table 2). The pVACs consist of roughly equal amounts of alpha- and beta-carotene (44-48% of total carotenoids). Sweet potatoes and Fe’i bananas have higher proportions of beta-carotene (60-90%) (Englberger et al. 2006). Using the 1:12 bioconversion ratio, a regular meal of 200 g of the plantain ‘Batard’ would appear to provide around a third of the daily vitamin A requirement for an average adult (500-900 µg/day), assuming that these pVACs are retained during processing.

Not only does the quantity and type of carotenoid influence the nutritional quality of foods, but other factors also have an effect:
• State of the food upon preparation (time in storage, ripeness, physical state),
• Age and physiological state of the consumer,
• The retention of pVACs in the food matrix (this relates to the digestibility of the food),
• The cooking or processing method,
• The other foods consumed at the same time.

Effect of ripening

In plantain, evidence suggests that the yellowing of the fruit pulp during ripening is caused by the breakdown of the chlorophyll, a process which reveals the carotenoids, rather than by carotenoid biosynthesis, as occurs in other fruits such as apricot, mango, papaya (Rodríguez-Amaya 1997). Giami and Alu (1994) found that total carotenoids in plantain almost halve during ripening. Similar trends were observed by Ngoh Newilah (2005), one of the collaborators in the HarvestPlus project, suggesting that the loss of beta-carotene in some micronutrient-rich varieties can be as high as 75%.

The present project is attempting to determine the point in fruit development at which carotenoid biosynthesis stops, which types of carotenoids are affected, the impact of letting the fruit ripen on the plant as opposed to ripening in storage, and how these vary according to the variety.

Plantains are cooked (e.g. fried, boiled, roasted, pureed) at various stages of ripeness depending on the maturity of the available fruit. For example, a surplus production may mean overripe plantain for breakfast, lunch and dinner. There is, however, evidence that the ripeness of plantain in processed meals is associated with the preferences of the consumer (Dury et al. 2002). If carotenoid content declines during ripening in many plantain varieties, then a change in storage and eating habits could deliver more micronutrients to the consumer.

Effect of cooking and processing

Cooking has contradictory effects on carotenoid levels. Processed foods may have higher levels of bioavailable carotenoids because of the loosening of the food matrix, allowing them to be more easily absorbed (Englberger et al. 2003, Van den Berg et al. 2000). On the other hand, cooking, especially under high heat and for a long time, destroys carotenoids, and converts trans isomers into cis isomers, which have lower vitamin A activity (Booth et al. 1992).

A report suggests a large percentage of carotenoids are retained in frying plantain (Rojas-Gonzalez et al. 2006). Furthermore, the levels of anti-nutrients in foods eaten at the same time, as well as their digestibility, influence the degree to which

Table 2. Available estimates of provitamin A carotenoid content and retinol activity in a selection of staple foods.

<table>
<thead>
<tr>
<th>Food</th>
<th>Beta-carotene equivalents (µg/g)</th>
<th>Retinol activity equivalents (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange-fleshed sweet potato</td>
<td>194&lt;sup&gt;1&lt;/sup&gt;</td>
<td>16</td>
</tr>
<tr>
<td>Utin lap (Fe’i type banana)</td>
<td>85&lt;sup&gt;2&lt;/sup&gt;</td>
<td>7.1</td>
</tr>
<tr>
<td>New strain ‘golden rice’</td>
<td>5&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Batard (plantain, AAB)</td>
<td>14&lt;sup&gt;3&lt;/sup&gt;</td>
<td>1.2</td>
</tr>
<tr>
<td>Cassava</td>
<td>7.7&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0.64</td>
</tr>
<tr>
<td>Cavendish dessert banana</td>
<td>1.4&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0.12</td>
</tr>
<tr>
<td>White rice</td>
<td>0&lt;sup&gt;5&lt;/sup&gt;</td>
<td>0</td>
</tr>
</tbody>
</table>

2 Englberger L. et al. 2006.
3 Coghlan A. New Scientist 27 March 2005. (reported as 37 mg of provitamin A - presumed to be beta-carotene equivalents)
micronutrients are absorbed and converted in the body. For instance, carotenoids are fat-soluble and evidence indicates that dietary fats in the meal facilitate the absorption of carotenoids (Yeum and Russell 2002).

In terms of micronutrient content, an exemplary meal might be plantain fried in red palm oil, one of the richest sources of carotenoids (Ngoh Newilah et al. 2005). The HarvestPlus project will examine more closely the effects on micronutrients of different traditional processing methods and practices, and the bioavailability of micronutrients to consumers.

Rather than focusing on the micronutrients in one variety, we could explore plantain- and banana-based subsistence systems as a whole. How do they function in terms of providing the full complement of nutrients required for a healthy diet? Which minerals or micronutrients are lacking or brought in from elsewhere?

**Delivering micronutrients to those who need them**

Tackling micronutrient deficiencies through improving the diet is clearly not just a question of identifying nutritious foods, but also of making such foods available in the quantities necessary to have an impact on health. The key question is whether the populations suffering from malnutrition have access to the micronutrient-rich foods intended for them.

Plantains and cooking bananas are subsistence crops in large parts of tropical Africa, including in areas where micronutrient deficiency has been identified as a problem. In Cameroon, for example, plantains form the major part of the diet almost everywhere. They are consumed in a multitude of ways, roasted, baked, fried, boiled, steamed, dried, pureed, or eaten raw (Ngoh Newilah et al. 2005). Few staple crops offer such versatility. In cities, however, plantain and cooking bananas are relatively expensive foods that are often out of reach for the urban poor. Reducing the price of plantain would require a substantial increase in year-round yields.

Yields of plantain and cooking banana in sub-Saharan Africa are notoriously low even though the technologies that could improve yields are tantalizingly simple; using clean planting material and encouraging denser planting are proving effective ways to increase production in trials. Any micronutrient-rich cultivars will need to be promoted together with production technologies that boost yields. For this reason, the HarvestPlus project is also carrying out on-farm trials on high-density production in Ghana and Cameroon.

The World Bank recently placed nutrition at the centre of the development agenda (World Bank, 2006). Agriculture and crop diversity clearly has an important role to play here. The HarvestPlus project, perhaps, represents yet another harbinger of a new era that demands research to consider its impact not just in terms of yields but in terms of health and well-being delivered.

**References**


Interactions between plant parasitic nematodes and plant secondary metabolism, with emphasis on phenylpropanoids in roots

Nathalie Wuyts

PhD thesis submitted in May 2006 to the Faculty of Bioscience Engineering, Katholieke Universiteit Leuven, Belgium

Plant parasitic nematodes impose a serious threat on agricultural production worldwide. Nematode resistant crops are generally considered the most favourable management option, as opposed to the much disputed use of chemical nematicides. For most crops, including banana, naturally resistant varieties are scarce or do not meet production or cultural standards. Knowledge on resistance mechanisms is still poor for many plant-nematode interactions, so breeding or genetic improvement techniques are not applied to their full capacity.

Plants produce a wide range of biologically active chemicals, secondary metabolites, which are involved in plant defence against pests and diseases. The major classes of secondary metabolites include alkaloids, terpenoids and phenylpropanoids. The biosynthetic pathway of the phenylpropanoids, the so-called phenolic compounds, is well-characterized and constitutes a potential target for the improvement of resistance against nematodes.

The objective of the present study was to gain a better understanding of the interaction between plant parasitic nematodes and plant secondary metabolites, in particular phenylpropanoids, in order to increase knowledge on plant defence against nematodes. The study was focused on the interaction between banana and its major nematode species *Radopholus similis*. Better knowledge of resistance mechanisms in banana and of the characteristic features of resistant varieties may facilitate breeding and screening of germplasm and hybrids, or provide a rationale for genetic improvement.

*In vitro* bioessays showed that secondary metabolites affect the behaviour of *Musa* nematodes, including *R. similis* and *Meloidogyne incognita*. Metabolites act as attractants or repellents, induce paralysis, reduce hatch or even cause death.

Five banana varieties with well-characterized host statuses for *R. similis*, including the susceptible ‘Grande naine’ (AAA, Cavendish subgroup) and ‘Obino l’ewai’ (AAB, plantain) and the resistant ‘Yangambi km 5’ (AAA, Ibota subgroup), ‘Pisang jari buaya’ (AA, Pisang jari buaya subgroup) and ‘Calcutta 4’ (*Musa acuminata* ssp. *burmannicoides*) were selected for the identification of potential physical and chemical barriers to nematode infection in banana roots. Methods included a quantitative lignin assay, liquid chromatography and mass spectrometry. Through histochemical staining phenylpropanoids were localized in root tissue.

Resistant banana varieties had more phenylpropanoids than susceptible ones. Cell walls of resistant roots contained significantly higher levels of lignin and ferulic acid esters. Lignin appeared to take part mainly in the protection of the vascular bundle both constitutively and upon infection. Ferulic acid esters in cortical cell walls act as substrates for peroxidase-catalysed dimerization and cross-linking of cell wall components and as initiation sites for lignification. Higher levels of these compounds in resistant varieties means that their cell walls are better equipped...
for modifications that increase resistance against hydrolytic enzymes secreted by nematodes during the infection process.

In general, resistant plants respond to infection by migratory endoparasitic nematodes, such as *R. similis*, with rapid and extensive—hypersensitive—tissue browning leading to non-expandable necrosis and an arrest in nematode migration. Tissue browning is the result of cellular damage and subsequent contact between oxidative enzymes, peroxidase and polyphenol oxidase, and their phenolic substrates. Banana roots contain an ample supply of substrate, dopamine, for polyphenol oxidase. In the resistant varieties, the levels of dopamine were higher than in susceptible ones. Besides dopamine, other compounds, which are probably related to anthocyanidin, were present in the roots and constitute potential chemical barriers to nematode infection.

Enzyme activity was assayed in roots of susceptible and resistant varieties infected with *R. similis*. First, it was found that dopamine and the phenylpropanoid catechin are the most efficient substrates for polyphenol oxidase extracted from banana roots. No positive correlation existed between the constitutive activity of phenylalanine ammonia-lyase (the first enzyme in the biosynthetic pathway of phenylpropanoids), peroxidase and polyphenol oxidase and resistance to *R. similis*. Nematode infection significantly induced phenylalanineammonia-lyase activity in the roots of the resistant variety ‘Yangambi km 5’.

The effect of the synthetic auxin transport inhibitor N-1-naphthylphthalamic acid on banana root development was also studied. The results indicate that N-1-naphthylphthalamic acid is effective at inducing physiological responses, such as a reduction in the number of nodal and lateral roots, a reduction in root length and loss of apical dominance. N-1-naphthylphthalamic acid can be used to study the potential interrelationship between banana root system development, nematode reproduction and auxin metabolism, and the role of phenylpropanoids and/or dopamine.

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**Characterization of a segregating population of *Musa* for parthenocarpy and male fertility**

*Frank Laban Turyagyenda*

**MSc thesis submitted in September 2005 to Makerere University, Uganda**

Parthenocarpy is the development of fruits in the absence of pollination and fertilization. The phenomenon offers several benefits to farmers, processing industries and consumers. However, parthenocarpic development tends to reduce chances of genetic improvement through cross breeding, because it is at times associated with reduced reproductive fertility. Most cultivated bananas are parthenocarpic and sterile. They are also susceptible to pests and diseases which are responsible for serious yield losses and threaten the crop. Resistance genes to most of these diseases and pests can be found in wild non-parthenocarpic diploids, which have high seed fertility. On crossing, most of the resulting hybrids inherit the inferior bunch characteristics of the wild diploid male parent. This therefore requires improving these wild diploids and their progenies for parthenocarpy before using them in resistance breeding. In bananas, the genetic basis of this important trait is complex and not fully understood, yet this knowledge is necessary for breeders to find a way of eliminating seed production in banana hybrids.

A number of cultivated triploid landraces have female fertility and can be genetically improved for disease and pest resistance by crossing them with resistant improved male parents. The rate of genetic improvement in cultivated *Musa* has been reported to depend on the reproductive fertility and the ability of male parents to produce viable pollen. Breeders are therefore interested in genotypes with good horticultural characteristics, resistance to pests and diseases and high male fertility for improving
cultivated triploids. The objectives of this study were to determine the number of genes controlling parthenocarpy in diploid Musa and evaluate pollen viability and agronomic performance of the hybrids to identify male parents suitable for breeding purposes.

Crosses were made between non-parthenocarpic TMB2x 6142-1 as the female parent and parthenocarpic TMB2x 8075-7 as the male parent. The resulting 89 offspring were established in the field and evaluated for parthenocarpy by bagging the emerging female inflorescence at anthesis. Pollen fertility of the progeny was evaluated by in vitro germination using a 3% sucrose solution medium.

Out of the 89 progenies, 69 had shrivelled and dried fruits and 20 had their fruits filled up with pulp. These results significantly fit a 1:3 ratio for three complementary genes controlling parthenocarpy. The analysis of variance suggests that genetic differences account for most of the phenotypic variation in parthenocarpy.

Eleven new genotypes namely TMB2x 2658S-20, TMB2x 2658S-35, TMB2x 2658S-45, TMB2x 2658S-58, TMB2x 2920S-5, TMB2x 2926S-1, TMB2x 2975S-6, TMB2x 2975S-11, TMB2x 2975S-40, TMB2x 2975S-44 and TMB2x 2975S-47, had both high pollen fertility rate and bunch weight, and were resistant to black Sigatoka. They are recommended as suitable male parents in banana breeding programmes to widen the genetic variability of the diploid male parent stock for black Sigatoka resistance and good agronomic traits. Pollen viability tests also showed that genotype influences physiological and biochemical processes involved in pollen grain germination. Further studies to determine physiological and biochemical processes related to pollen germination and tube growth in bananas are highly recommended.

Population dynamics, within-field and within-plant distribution of the banana skipper (Erionota thrax) (Lepidoptera: Hesperiidae) and its parasitoids in Penang, Malaysia

Nambangia Justin Okolle

PhD thesis submitted in April 2006 to the Universiti Sains, Malaysia

This research was aimed at studying the leaf-eating insect fauna, and the population dynamics and spatial distribution of a defoliating insect (Erionota thrax) and its major parasitoids in an intensively managed banana monoculture and in a low-input mixed subsistence farm.

The leaf-eating insects and their damage were sampled in two newly planted fields of ‘Pisang mas’, a local cultivar, and of a Cavendish commercial cultivar. The presence or absence of these insects was also recorded on the other crops and weeds. Between April and December 2004, eggs, larvae and pupae of E. thrax were sampled and reared in the laboratory to collect the parasitoids. The distribution and parasitism of E. thrax in relation to banana phenology and leaf age were recorded on preflowering plants, flowering plants, bunched plants, broad leaf followers, and narrow leaf followers.

Five insect species belonging to five families and three orders were recorded on both banana cultivars. Spodoptera litura was the most damaging on ‘Pisang mas’, causing more than 50% death in one to two months old plants, while E. thrax was the most damaging on Cavendish. E. thrax was not found on the weeds and other crops. The hymenopteran Ooencyrtus erionotae and Brachymeria albotibialis were the most important parasitoids of E. thrax eggs and pupae, with mean parasitism of 51.3%±5.8 and 38.6%±12.4, respectively. Infestation and parasitism of E. thrax were significantly higher on broad leaf followers and preflowering plants. Eggs and first instars were significantly more numerous on older leaves while older instars were more numerous on young leaves.
Postharvest characteristics and sensory evaluation of introduced FHIA and local 'Saba' varieties of banana

Edna Delas Alas Vida

PhD thesis submitted in 2005 to Cavite State University, the Philippines

The morphological, physico-chemical and physiological characteristics at harvest and at maturity of FHIA varieties from Honduras were compared to the ones of the local variety ‘Saba’. FHIA-03 was the most similar to ‘Saba’ in terms of bunch and fruit characteristics, ethylene production and respiration.

‘Saba’ was superior to all varieties in terms of fruit weight, girth and volume, as well as pulp firmness. FHIA-03 had the thickest peel. FHIA-23 had the heaviest bunch and highest moisture content of the pulp. The highest dry matter content was observed in FHIA-21.

The peel colour changed from green to yellow in all varieties. FHIA-23 took five days to ripen, ‘Saba’ and FHIA-21 seven and FHIA-03 nine, probably because of its very thick peel. FHIA-03 had the highest titrable acidity at harvest whereas FHIA-23 had the highest one at the ripe stage. There was no trace of total soluble solids when all the bananas were still green, but once they had ripened, FHIA-03 had the highest levels. Starch levels were highest in Saba and FHIA-21.

When ranking the varieties for banana chip and catsup production, panellists preferred FHIA-21 to ‘Saba’, whose chips were considered hard. The catsup made with FHIA-23 was the most preferred in terms of its taste, feel in the mouth, dark red colour and thick consistency.

Preparing for the battle against fusarium wilt

Fusarium wilt of banana, the notorious Panama disease that wiped out the plantations of Gros Michel export banana and led to its replacement by resistant Cavendish bananas in the second half of the twentieth Century is back. A new variant of the disease, dubbed Tropical Race 4, has been spreading through plantations of Cavendish bananas in Asia over recent years, reducing exports and raising the cost of production. The disease, caused by the fungus Fusarium oxysporum f. sp. cubense (Foc) has been successively reported in Taiwan, the Northern Territory of Australia, Indonesia (including Papua, formerly known as Irian Jaya), Malaysia, the southern provinces of China and, most recently, in the Philippines, the number one exporter of Cavendish bananas in Asia. The disease also threatens the traditional varieties that small-scale banana growers depend on for their livelihoods.

In preparation for meeting this threat, the Banana Asia-Pacific Network (BAPNET) teamed up with specialists from the Forestry and Agricultural Biotechnology Institute of South Africa (FABI), and The Queensland Department of Primary Industry and Fisheries (DP&F) to train plant pathologists to carry out surveys, identify infected plants, collect the fungi and identify the
vegetative compatibility groups (VCGs) into which the pathogen is classified. The International Fusarium Wilt Diagnosis and Characterization Training Workshop was held last April at the Malaysian Agricultural Research and Development Institute (MARDI) in Serdang, Malaysia. Twenty-five participants from Bangladesh, Cambodia, China, India, Indonesia, Malaysia, Philippines, Papua New Guinea, Sri Lanka, Thailand, Vietnam, Fiji, Taiwan, Costa Rica and Cuba attended the workshop.

Meanwhile, a project financed by the Australian Centre for International Agricultural Research (ACIAR) was launched in June to evaluate options to manage the disease. Often growers have no choice but to abandon an infected piece of land. Australian and Indonesian scientists have already been working together to look at alternatives, including biological control agents that can attack or compete with the fusarium pathogen in the soil. The new project will allow a wider range of options to be tested on farmers’ fields and lessons to be drawn for how to manage the disease wherever it occurs. One option will be to try resistant somaclonal variants developed in Taiwan. The various local cultivars in the collections of the Indonesian Tropical Fruits Research Institute (ITFRI) will also be evaluated for their resistance to the various VCGs found in Indonesia.

In addition to ITFRI, the project is conducted in collaboration with the Indonesian Agency for Agricultural Quarantine, the National Agricultural Research Institute (NARI) and National Agriculture Quarantine and Inspection Authority of Papua New Guinea, and DPI&F.

For more information, contact Dr. Augustin Molina at a.molina@cgiar.org
**Rescuing banana germplasm**

The Global Crop Diversity Trust and Bioversity International signed an agreement to support emergency activities to rehabilitate and secure germplasm maintained and conserved in the seed and field collections of the Institute of Plant Breeding – National Plant Genetic Resources Laboratory (IPB-NPGRL), which were damaged by the typhoon ‘Milenyo’ that hit the Philippines on 28 September. The agreement also covers funding and logistical support to repair damage and restore operations at IPB-NPGRL. This is the first time the Trust is intervening in an emergency situation. The Trust is an endowment fund managed by the FAO and the Consultative Group on International Agricultural Research to support the long-term conservation of vital food crops.

For more information, contact Jeffrey Oliver at j.oliver@cgiar.org

 Damage caused by the typhoon ‘Milenyo’ that hit the Philippines in September 2006.

**The new ProMusa**

As part of the process to revitalize ProMusa, an alliance with the International Society for Horticultural Science (ISHS) was formed establishing a new Section for Banana and Plantain. ProMusa was created in 1997 to provide support to Musa breeding through six interlinked working groups, each focusing on a particular subject: genetic improvement, fusarium wilt, Mycosphaerella leaf spot diseases, weevils, nematodes and viruses. Although ProMusa was perceived as a valuable forum for advancing research and addressing urgent questions, it was also felt that its operating mechanisms needed to change, to stimulate interaction among specialists and focus them on developing a coherent research-and-development agenda.

The new strategy and structure—three working groups on crop improvement, crop protection and crop production—focus on developing global public goods based on mobilizing the best science available internationally and bringing it to bear on the needs of the Musa research community in developing countries. The Crop Protection working group will hold its first global symposium “Recent advances in banana crop protection for sustainable production and improved livelihoods” in South Africa 10-14 September 2007. The proceedings will be published in ISHS’s Acta Horticulturae.

For more information on the meeting, visit ProMusa’s website at www.promusa.org or ISHS’s website at www.ishs.org.
**ISHS/ProMusa Symposium**

Recent advances in banana crop protection for sustainable production and improved livelihoods


**Programme**

Keynote lecture: Global challenges and opportunities in disease and pest management. Presented by Dr. David Jones

Session 1: Management of bacterial and viral diseases of banana
Session 2: New approaches to foliar disease management
Session 3: Enhancing soil health for pathogen and pest management
Session 4: Understanding diversity, managing diseases
Session 5: Understanding plant responses to disease and pest challenge
Session 6: Crop improvement strategies for pest and disease
Session 7: Improving crop protection on-farm

Keynote lecture: Managing diseases and pests of banana: The way ahead

Poster presentations
Field visit to banana farms

**Conference organizers**

Bioversity International
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**Conference fees**

US$480 for ISHS members
US$545 for non-members

Students get a reduction of US$50. The registration fee includes a copy of the proceedings of the symposium, which will be published in Acta Horticulturae, as well as a welcome reception, a conference dinner and all lunches. The additional cost for non-members includes a 12-month membership to ISHS. Among the many benefits, ISHS members can access free of charge up to 10 papers a year from any of the volumes of Acta Horticulturae available online.

Registration form is available at [www.promusa.org under 2007 symposium](http://www.promusa.org)
Recent publications
Celebrating 20 years of networking banana and plantain. INIBAP Annual Report 2005.
Developing a regional strategy to address the outbreak of banana Xanthomonas wilt in East and Central Africa. Proceedings of the banana Xanthomonas wilt regional preparedness and strategy development workshop held in Kampala, Uganda, 14-18 February 2005. E. Karamura, M. Osiru, G. Blomme, Ch. Lusty and C. Picq, editors. Proceedings of a workshop to develop a coherent regional and international response for containing the spread of banana Xanthomonas wilt in East and Central Africa and mitigating its impact on rural livelihoods.
Also available on this topic: The Banana Bacterial Wilt Resource CD which includes the most recent information about the disease, printable handouts and factsheets on how to recognize it and how to stop its spread, reports, selected literature and pictures and a searchable database.

Global conservation strategy for Musa (Banana and Plantain).
A consultative document prepared in collaboration with partners in the Musa research-and-development community. March 2006. This document presents a strategy to rationalize the conservation of the banana gene pool and promote the safe use and distribution of a wide range of diversity, all the way to farmers fields.

To obtain a complete list of our publications, consult our website or contact Leila Er-rachiq in Montpellier.
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