ISHS/ProMusa banana symposium

Global Perspectives on Asian Challenges

Phoenix City Hotel, Guangzhou, China
September 14-18, 2009

Programme and abstracts
This ISHS/ProMusa symposium is sponsored by the Guangdong Academy for Agricultural Sciences (GDAAS, China), Bioversity International (France/Italy), the Science and Technology Department of Guangdong Provincial Government (China) and the Agricultural Department of Guangxi Provincial Government (China).

The participation of delegates is supported by many organisations and individuals, without whose support this symposium would not have been possible. In this context, the Organising Committee would like to offer special thanks to the Technical Centre for Agricultural and Rural Cooperation (the Netherlands) that is supporting several participants from ACP countries, and the National Fund for Scientific Research (Belgium).

Numerous individuals and organisations generously contributed their time to the organisation of this symposium. The abstracts in this publication were edited by the members of the Editorial Committee. Special thanks go to Mike Smith, Huang Bingzhi, Rony Swennen, Nicolas Roux, Wei Yuerong, Catur Hermanto, Alice Churchill and Agustin Molina.

The contribution of all who have worked so hard towards the success of this meeting is gratefully acknowledged.
ISHS/ProMusa banana symposium

Global Perspectives on Asian Challenges

Phoenix City Hotel, Guangzhou, China
September 14-18, 2009

Programme and abstracts
## Programme

### Sunday, 13 September

<table>
<thead>
<tr>
<th>Arrival of participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:00-12:00 Registration</td>
</tr>
<tr>
<td>14:00-17:30 Registration</td>
</tr>
<tr>
<td>18:00 Welcome reception</td>
</tr>
</tbody>
</table>

### Monday, 14 September

<table>
<thead>
<tr>
<th>Arrival of participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>07:00-08:00 Registration</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Opening Session</th>
</tr>
</thead>
<tbody>
<tr>
<td>Session chair: Inge Van den Bergh</td>
</tr>
<tr>
<td>08:00-08:45 Welcome remarks</td>
</tr>
<tr>
<td>08:45-09:00 Group picture taking</td>
</tr>
<tr>
<td>09:00-09:30 Opening Keynote 1: Status, Challenges and Trends of the Chinese Banana Industry</td>
</tr>
<tr>
<td>G.J. Yi, C.Y. Li, Y.L. Wu, B.Z. Huang and Y.R. Wei</td>
</tr>
<tr>
<td>09:30-10:00 Opening Keynote 2: Using Musa Genes in the Fight against Production Constraints</td>
</tr>
<tr>
<td>E. Frison</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Session 1: Novel approaches to understanding, conserving and using banana genetic diversity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Session chairs: Huang Bingzhi and Mike Smith</td>
</tr>
<tr>
<td>10:00-10:30 Keynote 1: Non-Conventional Breeding of Banana (Musa spp.)</td>
</tr>
<tr>
<td>X.L. Huang, X. Huang, W. Xiao, J.T. Zhao, X.M. Dai, Y.F. Chen and Q. Gong</td>
</tr>
<tr>
<td>10:30-11:00 Coffee break</td>
</tr>
<tr>
<td>Time</td>
</tr>
<tr>
<td>--------------</td>
</tr>
<tr>
<td>11:00-11:30</td>
</tr>
<tr>
<td>11:30-11:45</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>11:45-12:00</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>12:00-12:15</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>12:15-12:30</td>
</tr>
<tr>
<td>12:30-14:00</td>
</tr>
<tr>
<td>14:00-14:15</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>14:15-14:30</td>
</tr>
<tr>
<td>14:30-14:45</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>14:45-15:00</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>15:00-15:15</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
15:15-15:30  Diversity Analysis in Indian Cooking Bananas (ABB) through Morphotaxonomic and Molecular Characterisation
   M.S. Saraswathi, S. Uma, E. Vadivel, P. Durai, S.A. Siva, G. Rajagopal and S. Sathiamoorthy

15:30-15:45  Application of Cytoplasmic and Nuclear DNA-Based Marker Systems for Elucidation of the Phylogenetic Relationship of Musa acuminata and Musa balbisiana and their Hybrids
   R. Boonruangrod, D. Desai, M. Berenyi, S. Fluch and K. Burg

15:45-16:00  Phylogenetic Relationships in the Family Musaceae Based on the Genic Sequences, Sequence of the ITS1-5.8S-ITS2 Region and DArT Markers
   P. Němcová, E. Hřibová, M. Valárik, J. Čížková, L. Schillerová, A. Kilian and J. Doležel

16:00-16:30  Coffee break

16:30-16:45  Molecular Analysis Reveals Multiple Domestications of Edible Bananas
   H. Volkaert

16:45-17:00  Microsatellite Markers for Classifying and Analysing Genetic Relationship of Banana Cultivars in Indonesia
   A. Retnoningsih, R. Megia and A. Hartana

17:00-17:15  Validation of Rapid (Colour-Based) Pre-Screening Techniques for Analysis of Fruit Provitamin A Contents in Musa spp.
   L.C. Pereira, G.N. Newilah, M.W. Davey and I. Van den Bergh

17:15-17:30  Micronutrients Biofortification in Musa: Status, Bottlenecks and Prospects
   M.W. Davey, J. Keulemans, N. Roux and I. Van den Bergh

17:30-18:30  Poster session 1

Tuesday, 15 September

Session 1: Novel approaches to understanding, conserving and using banana genetic diversity (continued)

Session chairs: Huang Bingzhi and Mike Smith

08:45-09:00  Candidate Gene Discovery: Resistance Gene Analog Characterisation and Differential Gene Expression Analysis in Musa-Mycosphaerella Host-Pathogen Interactions
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
</tr>
</thead>
</table>
| 09:00-09:15  | MA-ACS1: a Key Operator in Ethylene Biosynthesis in Banana - Its Role and Regulation during Fruit Ripening  
* S.R. Choudhury, S. Roy, S.K. Singh and D.N. Sengupta  |
| 09:15-09:30  | Combination of Suppression-Subtractive Hybridisation with cDNA Microarray, a Novel Way to Identify Genes from Banana Involved in Fruit Ripening, Quality Improvement and Tolerance to Stress  
* Z.Q. Jin and B.Y. Xu  |
| 09:30-09:45  | Developing Resistant Banana and Plantain Cultivars through Conventional Breeding Techniques  
* R. Menon, A. Cherian, A. Suma, A. Maicykutty, P. Mathew, S. Nair and K.C. Aipe  |
| 09:45-10:00  | Exploitation of Diploids in Indian Banana-Breeding Programmes  
* S. Uma, M.M. Mustaffa, M.S. Saraswathi and P. Durai  |
| 10:00-10:15  | Breeding Increases Genetic Diversity of East African Highland Bananas (*Musa* spp.): an Assessment Using Molecular Tools  
* N. Moses and M. Pillay  |
| 10:15-10:30  | Use of Molecular Markers in Banana and Plantain Improvement  
* J. Lorenzen, S. Hearne, G. Mbanjo, M. Nyine and T. Close  |
| 10:30-11:00  | Coffee break  |
| 11:00-11:15  | The Use of Molecular Markers in CIRAD’s Current Banana Breeding Programme  
* J-P. Horry  |
| 11:15-11:30  | Development of Highly Regenerative Embryogenic Cell Suspensions of Banana Cultivar ‘Nanjangud Rasbale’ (syn. ‘Rasthali’, AAB, Silk Subgroup) and Transformants with *AMP* Gene  
* S. Mohandas, H.D. Sowmya, R. Manjula, K.Y. Pratibha, M. Manamohan and S. Meenakshi  |
<p>| 11:30-12:30  | Discussion  |
| 12:30-14:00  | Lunch  |</p>
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
</tr>
</thead>
</table>
| 14:00-14:30| Keynote 1: Studies on Some of the Early Events in the *Fusarium oxysporum-Musa* Interaction  
| 14:30-15:00| Keynote 2: Fusarium Wilt of Banana: Renewed Threat and Renewed R&D Interest  
A.B. Molina |
| 15:00-15:15| Incidence and Distribution of Fusarium Wilt in Indonesia  
C. Hermanto, A. Sutanto, Jumjunidang, H.S. Edison, J. Daniells, W. O'Neill, V.G. Sinohin and A.B. Molina |
| 15:15-15:30| Status of Fusarium Wilt Research in India  
M.M. Mustaffa and R. Thangavelu |
| 15:30-15:45| Raising Awareness of the Threat of Tropical Race 4 of Fusarium Wilt for Latin America and the Caribbean  
L.E. Pocasangre, R. Ploetz, A.B. Molina and L. Perez Vicente |
| 15:45-16:00| Development of a Detection Method for Tropical Race 4 of *Fusarium oxysporum* f. sp. *cubense*  
| 16:00-16:30| Coffee break |
| 16:30-16:45| Characterisation of Isolates of *Fusarium oxysporum* f. sp. *cubense* into Vegetative Compatibility Groups in Brazil  
| 16:45-17:00| Vegetative Compatibility Group Analysis of Indonesian *Fusarium oxysporum* f. sp. *cubense* Isolates  
Cross Infection Potential of Fusarium Wilt Isolates and their Diversity Analysis by Vegetative Compatibility Grouping, Sequencing of rDNA-ITS region and rDNA-IGS- RFLP Analysis

R. Thangavelu, P. Suganya Devi and P. Maria Chrismala (presented by M.M. Mustaffa)

Gene Expression Analysis in Roots of Musa acuminata ssp. burmannicoides ‘Calcutta 4’, a Resistant Genotype for Fusarium Wilt in Banana

K.V. Ravishankar, A. Rekha, V. Swarupa and G. Savitha

Application of gfp-Transformed Fusarium oxysporum f. sp. cubense for the Tracking of the Infection Process and the Evaluation of Resistant Banana Cultivars


Characterisations of Early Events in the Fluorescent-Tagged Fusarium oxysporum f. sp. cubense and Banana Root Interaction

X.M. Yin, W. Zheng, B.Y. Xu, J.B. Wang ZhiQiang Jin

Wednesday, 16 September

Session 2: Integrated approaches to managing Fusarium wilt and other emerging disease threats (continued)

Session chairs: Catur Hermanto and Wei Yuerong

A Greenhouse Bio-Assay for the Fusarium oxysporum f. sp. cubense (Tropical Race 4) x Banana (Cavendish Subgroup) Interaction


Discrimination of Banana Genotypes for Fusarium Wilt Resistance in Greenhouse


Reaction of Diploid (AA) and Tetraploid (AAAB) Banana Hybrids to Fusarium Wilt under Field Conditions

A.P. de Matos, Z.J.M. Cordeiro, S. de Oliveira e Silva and D.M.V. Ferreira

Fusarium Wilt Management through Use of Resistant Genotypes, Chemical and Biological Approaches

A. Cherian, P.M. Mathew, R. Menon, A. Suma, K.C. Aipe and S.N. Nair

Selection of a New Somaclone Cultivar ‘Tai-Chiao No.5’ with Resistance to Fusarium Wilt of Banana in Taiwan

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
</tr>
</thead>
</table>
| 10:00-10:15  | Comparison of Host Reaction to *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 and Agronomic Performance of Somaclonal Variant ‘GCTCV 119’ and ‘Grand Naine’ in Commercial Farms in the Philippines  
E.T. Fabregar, R.O. Soquita, V.S. Sinohin and A.B. Molina |
| 10:15-10:30  | Combating Banana Wilts – What Do Resistant Cultivars Have to Offer?     
*J.W. Daniells* |
| 10:30-11:00  | **Coffee break**                                                        |
| 11:00-11:15  | Fusarium Wilt Incidence, Growth, Yield and Post-Harvest Quality of Banana as Affected by Organic Farming System in Taiwan  
*C.M. Chang, C.P. Chao, S.N. Huang and S.C. Chiang* |
| 11:15-11:30  | Efficacy of Clay-Based Formulated *Serratia* in Reducing Inoculum of *Fusarium oxysporum* f. sp. *cubense* Race 4  
*A.S.Y. Ting, M.T. Fang and C.S. Tee* |
| 11:30-11:45  | Genetic Structure of *Mycosphaerella fijiensis* Populations in Costa Rica  
| 11:45-12:00  | Transcriptome Analysis of *Mycosphaerella fijiensis*, the Causal Agent of Black Leaf Streak Disease in Banana  
| 12:00-12:15  | Bridging the Technology Gap in Banana Improvement: The Case of Biotechnology Adoption by Developing Countries  
*M.N. Mwangi* |
| 12:15-12:30  | Characterisation of the Digestive Proteases in the Banana Weevil (*Cosmopolites sordidus*) Gut and the Effects of Recombinant Phytocystatins and Bt Cry6A on Early Larval Growth and Development  
*A. Kiggundu, K. Kunert, D. Michaud, A. Viljoen, W. Tushemereirwe and E. Karamura* |
| 12:30-14:00  | **Lunch**                                                                |
| 14:00-14:15  | Screening 40 *Musa* Genotypes for *Banana Bunchy Top Virus* Resistance in Burundi  
*C. Niyongere, E. Miinda Ateka, T. Losenge, P. Lepoint and G. Blomme* |
| 14:15-14:30  | *Banana Bunchy Top Virus*-Resistant Transgenic Banana Plants  
*W. Borth, E. Perez, K. Cheah, Y. Chen, W.S. Xie, D. Gaskill1, S. Khalil and J.S. Hu* |
| 14:30-14:45  | Management of Banana Diseases and Pests by Use of Tissue Culture-Derived Planting Material in Kenya  
*J. Njuguuna, S. Nguthi, F. Wambugu, D. Gitau and M. Karuoya* |
14:45-15:00  Virus-Indexing Technology in Banana: A Boon to the Tissue-Culture Industries and Banana Growers for Production of Quality Planting Material in India  
*R. Selvarajan*, V. Balasubramanian, M. Mary Sheeba, R. Raj Mohan and M.M. Mustaffa

15:00-16:00  Discussion

16:00-16:30  Coffee break

### Closing Session

Session chair: Zaag de Beer

16:30-17:00  Closing Keynote 1: Harnessing the Potential of Banana and Plantain in Asia and the Pacific for Inclusive Growth  
*H.P. Singh*

17:00-17:30  Closing Keynote 2: Making Science Relevant - Linking Agricultural Research Networks to Innovation Platforms  
*S. Weise*

17:30-17:45  Closing remarks: *I. Van den Bergh*

19:30  Gala dinner

- Announcement of election results for the position of chair and vice-chair of the ISHS Section on Banana and Plantain

### Thursday, 17 September

**Field trip**

08:00-23:00  Visit to various places, such as the Guangdong Academy to see their banana collection, Panyo to see traditional Chinese architecture, the banana production area in Zhongshan, and Zhuhai for cultural entertainment and dinner

### Friday, 18 September

**Workshop 1: The role of genomics in banana crop improvement**

Facilitators: Huang Xuelin, Nicolas Roux and Mike Smith

09:00-09:30  Opening of Workshop 1: Opportunities for Bridging the Gap between Genomics and Genetic Improvement in *Musa* spp.  
*N. Roux, M. Smith, M. Rouard, X.L. Huang and the GMGC consortium*
<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:30-10:30</td>
<td>Workshop</td>
</tr>
<tr>
<td>10:30-11:00</td>
<td>Coffee break</td>
</tr>
<tr>
<td>11:00-12:30</td>
<td>Workshop</td>
</tr>
<tr>
<td>12:30-14:00</td>
<td>Lunch</td>
</tr>
<tr>
<td>14:00-15:30</td>
<td>Workshop</td>
</tr>
<tr>
<td>15:30-16:00</td>
<td>Coffee break</td>
</tr>
</tbody>
</table>

**Workshop 2: Mitigating the threat of Fusarium wilt**

Facilitators: Altus Viljoen, Randy Ploetz and Alice Churchill

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
</tr>
</thead>
</table>
| 09:00-09:30  | Opening of Workshop 2: Fusarium wilt – A Banana Disease that Refuses to Go Away  
* R. Ploetz, A. Churchill and A. Viljoen |
| 09:30-10:30  | Workshop                                      |
| 10:30-11:00  | Coffee break                                 |
| 11:00-12:30  | Workshop                                      |
| 12:30-14:00  | Lunch                                         |
| 14:00-15:30  | Workshop                                      |
| 15:30-16:00  | Coffee break                                 |

**Reporting back to plenary**

Session chairs: Inge Van den Bergh and Zaag de Beer

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:00-16:30</td>
<td>Report from workshop 1</td>
</tr>
<tr>
<td>16:30-17:00</td>
<td>Report from workshop 2</td>
</tr>
</tbody>
</table>
| 17:00-18:00  | Discussion about ISHS-ProMusa symposia 2010-1012  
Any other business, Open floor |
Abstracts

Oral Presentations

Opening Session

Opening Keynote 1: Status, Challenges and Trends of the Chinese Banana Industry
G.J. Yi, C.Y. Li, Y.L. Wu, B.Z. Huang and Y.R. Wei ................................................................. 3

Opening Keynote 2: Using Musa Genes in the Fight against Production Constraints
E. Frison ........................................................................................................................................ 4

Session 1: Novel Approaches to Understanding, Conserving and Using Banana Genetic Diversity

Keynote 1: Non-Conventional Breeding of Banana (Musa spp.)
X.L. Huang, X. Huang, W. Xiao, J.T. Zhao, X.M. Dai, Y.F. Chen and Q. Gong .................... 5

Keynote 2: From Fundamental Research Discoveries to Applications for Banana Improvement

Genomics, Banana Breeding and Superdomestication
J.S. Heslop-Harrison .................................................................................................................. 7

Analysis of Genome Structure and Organisation in Banana (Musa acuminata) Using 454 Sequencing
E. Hřibová, P. Neumann, J. Macas and J. Doležel ...................................................................... 8

Genome-Wide BAC End Sequencing of Musa acuminata ‘DH Pahang’ Reveals Further Insights into the Genome Organisation of Banana
M.T. Souza Jr, N. Roux and G.H.J. Kema 3 .................................................................................. 9

Identification and Validation of EST-Derived Molecular Markers, TRAP and VNTR, for Banana Research

Wild Musa species in China
M. Häkkinen .................................................................................................................................. 11

Musa Collection and Characterisation in Central and Eastern DR-Congo: a Chronological Overview
B. Dheda Djailo, B.D. Nzawele, N. Roux, F. Ngezahayo, N. Vigheri, E. De Langhe,
D. Karamura, S. Channelière, M. Ruas, C. Picq and G. Blomme ................................................ 12
Table of Contents

Morphological Characterisation of East African AAB and AA Dessert Bananas (*Musa* spp.)
*M. Onyango, D. Karamura, S. Keeley, R. Manshardt and D. Haymer*................................................. 13

The Complementarity of Farmers’ and Botanical Descriptors of the East African Highland Banana Cultivars (*Musa*, AAA)
*D. Karamura, A. Kiggundu and E. Karamura* ................................................................................................ 14

Standardised Procedures for *Musa* Germplasm Characterisation – Use of a Reference Collection – Towards a *Musa* Identification Key?
*S. Channelière, A. Vezina, E. Arnaud, J-P. Horry, M. Ruas and N. Roux*.............................................. 15

Diversity Analysis in Indian Cooking Bananas (ABB) through Morphotaxonomic and Molecular Characterisation
*M.S. Saraswathi, S. Uma, E. Vadivel, P. Durai, S.A. Siva, G. Rajagopal and S. Sathiamoorthy* .... 16

Application of Cytoplasmic and Nuclear DNA-Based Marker Systems for Elucidation of the Phylogenetic Relationship of *Musa acuminata* and *Musa balbisiana* and their Hybrids
*R. Boonruangrod, D. Desai, M. Berenyi, S. Fluch and K. Burg*................................................................. 17

Phylogenetic Relationships in the Family Musaceae Based on the Genic Sequences, Sequence of the ITS1-5.8S-ITS2 Region and DArT Markers
*P. Němcová, E. Hřibová, M. Valárik, J. Čížková, L. Schillerová, A. Kilian and J. Doležel*.......... 18

Molecular Analysis Reveals Multiple Domestications of Edible Bananas
*H. Volkaert* ................................................................................................................................................ 19

Microsatellite Markers for Classifying and Analysing Genetic Relationship of Banana Cultivars in Indonesia
*A. Retnoningsih, R. Megia and A. Hartana*................................................................................................. 20

Validation of Rapid (Colour-Based) Pre-Screening Techniques for Analysis of Fruit Provitamin A Contents in *Musa* spp.
*L.C. Pereira, G.N. Nevilah, M.W. Davey and I. Van den Bergh*.............................................................. 21

Micronutrients Biofortification in *Musa*: Status, Bottlenecks and Prospects
*M.W. Davey, J. Keulemans, N. Roux and I. Van den Bergh*................................................................. 22

Candidate Gene Discovery: Resistance Gene Analog Characterisation and Differential Gene Expression Analysis in *Musa-Mycosphaerella* Host-Pathogen Interactions

MA-ACS1: a Key Operator in Ethylene Biosynthesis in Banana - Its Role and Regulation during Fruit Ripening
*S.R. Choudhury, S. Roy, S.K. Singh and D.N. Sengupta* .......................................................................... 24
Combination of Suppression-Subtractive Hybridisation with cDNA Microarray, a Novel Way to Identify Genes from Banana Involved in Fruit Ripening, Quality Improvement and Tolerance to Stress  
**Z.Q. Jin and B.Y. Xu** ...........................................................................................................................25

Developing Resistant Banana and Plantain Cultivars through Conventional Breeding Techniques  
**R. Menon, A. Cherian, A. Suma, A. Maicykutty, P. Mathew, S. Nair and K.C. Aipe** .....................26

Exploitation of Diploids in Indian Banana-Breeding Programmes  
**S. Uma, M.M. Mustaffa, M.S. Saraswathi and P. Durai** .................................................................27

Breeding Increases Genetic Diversity of East African Highland Bananas (*Musa* spp.): an Assessment Using Molecular Tools  
**N. Moses and M. Pillay** .......................................................................................................................28

Use of Molecular Markers in Banana and Plantain Improvement  
**J. Lorenzen, S. Hearne, G. Mbanjo, M. Nyine and T. Close** ...............................................................29

The Use of Molecular Markers in CIRAD’s Current Banana Breeding Programme  
**J-P. Horry** ...........................................................................................................................................30

Development of Highly Regenerative Embryogenic Cell Suspensions of Banana Cultivar ‘Nanjungad Rasbale’ (syn. ‘Rashthali’, AAB, Silk Subgroup) and Transformants with AMP Gene  
**S. Mohandas, H.D. Sowmya, R. Manjula, K.Y. Pratibha, M. Manamohan and S. Meenakshi** ........31

Opening of Workshop 1: Opportunities for Bridging the Gap between Genomics and Genetic Improvement in *Musa* spp.  
**N. Roux, M. Smith, M. Rouard, X.L. Huang and the GMGC consortium** ........................................32

**Session 2: Integrated Approaches to Managing Fusarium Wilt and Other Emerging Disease Threats** ..........................................................................................................................33

Keynote 1: Studies on Some of the Early Events in the *Fusarium oxysporum-Musa* Interaction  
**C.Y. Li, G.J. Yi, S. Chen, Q.M. Sun, C.W. Zuo, B.Z. Huang, Y.R. Wei, Y.H. Huang, Y.L. Wu, L.B. Xu and C.H. Hu** ........................................................................................................................................33

Keynote 2: Fusarium Wilt of Banana – Renewed Threat and Renewed R&D Interest  
**A.B. Molina** .........................................................................................................................................34

Incidence and Distribution of Fusarium Wilt in Indonesia  
**C. Hermanto, A. Sutanto, Jumjunidang, H.S. Edison, J. Daniells, W. O’Neill, V.G. Sinohin and A.B. Molina** ..................................................................................................................................................35

Status of Fusarium Wilt Research in India  
**M.M. Mustaffa and R. Thangavelu** .....................................................................................................36
Table of Contents

Raising Awareness of the Threat of Tropical Race 4 of Fusarium Wilt for Latin America and the Caribbean
L.E. Pocasangre, R. Ploetz, A.B. Molina and L. Perez Vicente ...................................................... 37

Development of a Detection Method for Tropical Race 4 of Fusarium oxysporum f. sp. cubense

Characterisation of Isolates of Fusarium oxysporum f. sp. cubense into Vegetative Compatibility Groups in Brazil

Vegetative Compatibility Group Analysis of Indonesian Fusarium oxysporum f. sp. cubense Isolates

Cross-Infection Potential of Fusarium Wilt Isolates and their Diversity Analysis by Vegetative Compatibility Grouping, Sequencing of rDNA-ITS region and rDNA-IGS- RFLP Analysis
R. Thangavelu, P. Suganya Devi and P. Maria Chrismala (presented by M.M. Mustaffa) ......... 41

Gene Expression Analysis in Roots of Musa acuminata ssp. burmannicoides ‘Calcutta 4’, a Resistant Genotype for Fusarium Wilt in Banana
K.V. Ravishankar, A. Rekha, V. Swarupa and G. Savitha ................................................................. 42

Application of gfp-Transformed Fusarium oxysporum f. sp. cubense for the Tracking of the Infection Process and the Evaluation of Resistant Banana Cultivars

Characterisations of Early Events in the Fluorescent-Tagged Fusarium oxysporum f. sp. cubense and Banana Root Interaction

A Greenhouse Bio-Assay for the Fusarium oxysporum f. sp. cubense (Tropical Race 4) x Banana (Cavendish Subgroup) Interaction

Discrimination of Banana Genotypes for Fusarium Wilt Resistance in Greenhouse

Reaction of Diploid (AA) and Tetraploid (AAAB) Banana Hybrids to Fusarium Wilt under Field Conditions
A.P. de Matos, Z.J.M. Cordeiro, S. de Oliveira e Silva and D.M.V. Ferreira ................................. 47

Fusarium Wilt Management through Use of Resistant Genotypes, Chemical and Biological Approaches
A. Cherian, P.M. Mathew, R. Menon, A. Suma, K.C. Aipe and S.N. Nair ....................................... 47

iv
# Table of Contents

Selection of a New Somaclone Cultivar ‘Tai-Chiao No.5’ with Resistance to Fusarium Wilt of Banana in Taiwan  

Comparison of Host Reaction to *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 and Agronomic Performance of Somaclonal Variant ‘GCTCV 119’ and ‘Grand Naine’ in Commercial Farms in the Philippines  
*E.T. Fabregar, R.O. Soquita, V.S. Sinohin and A.B. Molina* .............................................................49

Combating Banana Wilts – What Do Resistant Cultivars Have to Offer?  
*J.W. Daniells* .......................................................................................................................................50

Fusarium Wilt Incidence, Growth, Yield and Post-Harvest Quality of Banana as Affected by Organic Farming System in Taiwan  
*C.M. Chang, C.P. Chao, S.N. Huang and S.C. Chiang* ........................................................................50

Efficacy of Clay-Based Formulated *Serratia* in Reducing Inoculum of *Fusarium oxysporum* f. sp. *cubense* Race 4  
*A.S.Y. Ting, M.T. Fang and C.S. Tee* ..................................................................................................51

Genetic Structure of *Mycosphaerella fijiensis* Populations in Costa Rica  

Transcriptome Analysis of *Mycosphaerella fijiensis*, the Causal Agent of Black Leaf Streak Disease in Banana  

Bridging the Technology Gap in Banana Improvement: The Case of Biotechnology Adoption by Developing Countries  
*M.N. Mwangi* .......................................................................................................................................55

Characterisation of the Digestive Proteases in the Banana Weevil (*Cosmopolites sordidus*) Gut and the Effects of Recombinant Phytocystatins and Bt Cry6A on Early Larval Growth and Development  
*A. Kiggundu, K. Kunert, D. Michaud, A. Viljoen, W. Tushemereirwe and E. Karamura* ..................56

Screening 40 *Musa* Genotypes for *Banana Bunchy Top Virus* Resistance in Burundi  
*C. Niyongere, E. Miinda Ateka, T. Losenge, P. Lepoint and G. Blomme* .............................................57

*Banana Bunchy Top Virus*-Resistant Transgenic Banana Plants  
*W. Borth, E. Perez, K. Cheah, Y. Chen, W.S. Xie, D. Gaskill, S. Khalil and J.S. Hu* .........................58

Management of Banana Diseases and Pests by Use of Tissue Culture-Derived Planting Material in Kenya  
*J. Njuguna, S. Nguthi, F. Wambugu, D. Gitau and M. Karuoya* .......................................................59
Table of Contents

Virus-Indexing Technology in Banana: A Boon to the Tissue-Culture Industries and Banana Growers for Production of Quality Planting Material in India
R. Selvarajan, V. Balasubramanian, M. Mary Sheeba, R. Raj Mohan and M.M. Mustaffa ........... 60

Opening of Workshop 2: Fusarium wilt – A Banana Disease that Refuses to Go Away
R. Ploetz, A. Churchill and A. Viljoen ................................................................................................. 61

Closing Session .................................................................................................................................... 63

Closing Keynote 1: Harnessing the Potential of Banana and Plantain in Asia and the Pacific for Inclusive Growth
H.P. Singh ................................................................................................................................................. 63

Closing Keynote 2: Making Science Relevant - Linking Agricultural Research Networks to Innovation Platforms
S. Weise .................................................................................................................................................... 64

Poster Presentations ............................................................................................................................. 67

Session 1: Novel Approaches to Understanding, Conserving and Using Banana Genetic Diversity .................................................................................................................................................. 69

Musa Collection, Characterisation and Improvement in China

Banana Germplasm Investigation, Collection and Conservation in Guangxi, China
J.Y. Yao, X.Q. Qin, X. Long, H.X. Peng ................................................................................................. 70

Preliminary Study on ISSR Analysis and Classification of Wild Banana (Musa spp.) in Guangxi, China
X.Q. Qin, H.X. Peng, X. Long and J.Y. Yao .......................................................................................... 71

Analysis of Genetic Diversity in Banana (Musa spp.) using Sequence-Related Amplified Polymorphism Markers
J.Y. Wei, D.B. Liu, S.X. Wei, Z.S. Xie, G.Y. Xu and Y.Y. Chen ..................................................................... 72

Study of Banana Germplasm by AFLP Based on the Technique of Capillary Electrophoresis
J.W. Zeng, X. Long, R. Xia, B.Z. Huang, Y.H. Huang, G.J. Yi ............................................................... 73

Studies on Intersectional Relationship between Eumusa and Rhodochlamys of the Genus Musa Using Morphotaxonomy and Microsatellite Markers
P. Durai, S. Uma, M.S. Saraswathi, N. Jayabalun and M.M. Mustaffa .................................................. 74

Robustness of IRAP and RAPD Marker Systems in Studying the Intra-Group Diversity of Musa Cavendish (AAA) clones
M.S. Saraswathi, S. Uma, K. Prasanya Selvam, S. Ramaraj, P. Durai and M.M. Mustaffa ........... 75
<table>
<thead>
<tr>
<th>Title</th>
<th>Authors</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Studies on Genetic Resources of Seeded Banana in West Bengal</td>
<td>M.A. Hasan, R. Ray Chowdhury, M. Manna, K.K. Mandal, D. Majumder and S. Jha</td>
<td>76</td>
</tr>
<tr>
<td>Generation of Mapping Populations for Segregation of ‘B’ Genome</td>
<td>A. Rekha, K.V. Ravishankar and D.S. Ambika</td>
<td>77</td>
</tr>
<tr>
<td>Genotyping of Dessert Banana Cultivar ‘Kolikuttu’ (AAB, Silk)</td>
<td>W.L.G. Samarasinghe, H.W.L. Pushpakumari, J.L.P. De Silva and S.G.J.N. Senanayake</td>
<td>78</td>
</tr>
<tr>
<td>Genetic Structure of Musa acuminata (AA) Populations in Sri Lanka</td>
<td>W.L.G. Samarasinghe and S.L.D. Jayaweera</td>
<td>78</td>
</tr>
<tr>
<td>Post-Harvest Characterisation of Three Banana Cultivars from the</td>
<td>G. Ngoh Newilah, C. Dhuique-Mayer, K. Tomekpe, E. Fokou and F.X. Etoa</td>
<td>79</td>
</tr>
<tr>
<td>CARBAP Musa Germplasm Collection in Cameroon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Determination of Nutritional Composition of Four Banana Cultivars</td>
<td>M.A.L.N. Mallawaarachchi</td>
<td>80</td>
</tr>
<tr>
<td>available in the Market of Up-Country Intermediate Zone of Sri Lanka</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruit Quality of Some Banana Cultivars Grown in Tunisian Coastal</td>
<td>A. Ferchichi, M. Ben Salah and M. Jeridi</td>
<td>81</td>
</tr>
<tr>
<td>Oases of Gabes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expression of MaMADS2 and Its Interactions with Ethylene Suggest that</td>
<td>T. Elitzur, E.E. Goldschmidt, J. Giovannoni, J. Vrebalov and H. Friedman</td>
<td>82</td>
</tr>
<tr>
<td>itActs Upstream to Ethylene Production</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flower Bud from Two Chinese Banana Cultivars, ‘Baxijiao’ (AAA) and</td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Paradisical’ (AAB)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roles of Soluble Sugars on Degreening of Banana Fruit</td>
<td>L.Y. Xu, X.T. Yang, Z.Q. Zhang, R.Q. Fang and X.Q. Pang</td>
<td>84</td>
</tr>
<tr>
<td>Food Attributes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sequence Comparison of cDNAs and Expression Analysis of Phenylalanine</td>
<td>J. Correa, A. Rodríguez, E. Rodríguez-Arango, Z.I. Monsalve and R. Arango</td>
<td>86</td>
</tr>
<tr>
<td>Ammonia-Lyase from Different Banana Cultivars after Infection with</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mycosphaerella fijiensis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gene Expression Analysis in Leaves of ‘Bee Hee Kela’, a Drought</td>
<td>K.V. Ravishankar, A. Rekha, R.H. Laxman, G. Savitha and V. Swarupa</td>
<td>87</td>
</tr>
<tr>
<td>Tolerant Banana Genotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pratylenchus coffeae for Creation of Subtractive cDNA Library</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table of Contents

Isolation of a Cyclin D2;1-type Gene Homologue from East African Highland Banana (Musa, AAA)
D. Talengera, A. Kiggundu, W.K. Tushemereirwe, D. Inze and K. Kunert ........................................ 89

Seed as an Alternative Source of DNA for Molecular Research of Inaccessible Wild Musa species
S. Uma, M.S. Saraswathi and D. Anto .................................................................................................. 90

Role of In-Vitro Selection of Multiple Bud Clumps in the Screening of Fusarium Wilt Resistant Somaclones of Bananas in Taiwan
S.W. Lee, S.Y. Lee and M.J. Huang ........................................................................................................ 90

In-Vitro Mutagenesis for Banana Improvement
S. Mohan Jain ........................................................................................................................................ 91

Synchronisation of Somatic Embryos by Liquid Medium-Based Protocol for ‘Rasthali’ (Musa, AAB)
S. Uma, A. Akbar, M. S. Saraswathi, and K. Udhayaanjali ................................................................. 92

Histological Analysis of Somatic Embryogenesis in the Banana Cultivar ‘Mas’ (AA)
Y.R. Wei, G.J. Yi, C.H. Hu, B.Z. Huang, C.Y. Li and X.L. Huang ....................................................... 93

Genetic Transformation of GUS Gene into the Banana Cultivar ‘Pisang Mas’ (AA) by Agrobacterium-Mediated Transformation
C.H. Hu, Y.R. Wei and G.J. Yi ................................................................................................................. 94

Production of Suitable Target Tissues for Banana Transformation Studies
M. Maziah, S. Sreeramanan and M. Sariah ............................................................................................ 95

Use of an Improved Site-Directed Mutagenesis for Creation of Constructs to Analyse Gene Function in Fusarium oxysporum f. sp. cubense

Development of Highly Regenerative Embryogenic Cell Suspensions and Transformants with AMP Gene of Banana Cultivar ‘Nanjangud Rasbale’ (syn. ‘Rasthali’, AAB, Silk subgroup)
S. Mohandas, H.D. Sowmya, R. Manjula, K.Y. Pratibha, M. Manamohan and S. Meenakshi ........... 97

Improving Regeneration of Transformed Banana by Reducing Explant Browning
J.Y. Li, J.B. Zhang, B.Y. Xu and Z.Q. Jin ............................................................................................... 98

New Cultivars, New Options: The Potential of Introduced Bananas

Distribution of Commercial Cultivars, Landraces and Wild Musa in Indonesia
## Table of Contents

Alternative Ex-Situ Conservation Strategy to Ensure Virus-Free Status and True-to-Typeness of *Musa* Germplasm  

Status of *Musa* Genetic Conservation in India  
**H.P. Singh** ..................................................................................................................101

In-Vitro Conservation and Cryopreservation of Genetic Resources of *Musa* in India: A Network Approach  
**A. Agrawal, R.K. Tyagi and S.K. Sharma** .......................................................................103

Conservation, Evaluation and Utilisation of Introduced *Musa* Germplasm in Kerala, India  
**R. Menon, A. Cherian, A. Suma and S. Nair** .................................................................104

Evaluation and Tissue-Culture Conservation, Multiplication and Distribution of Rare and Carotenoid-Rich Fe’i Banana Cultivars in Micronesia  
**V.M. Verma** ...................................................................................................................105

Micropropagation of Malaysian Banana Cultivars (*Musa* spp.) Using a Modified Twin-Flask System  
**L.K. Chan and V.H. Au** ..................................................................................................105

Cryopreservation of In-Vitro Shoot Tips of *Musa* Germplasm by Droplet Vitrification  
**J.G. Li, S.M. Zhang, H.B. Chen, C.X. Xu and Z.H. Wang** ..................................................106

Effect of Cytokinins on the Proliferation Rate of Banana (*Musa* spp.) Plantlets  
**M. Maziah, F. Mahdavi, M. Sariah, M.P. Puad and S. Shirani** ...........................................107

The Effect of Different Cytokinins on Plant Regeneration from Male Flowers of Banana  
**F. Mahdavi, M. Maziah, M. Sariah, M.P. Puad and S. Shirani** ............................................108

Effects of Cytokinins on Proliferation Rate and Abnormality Index of Banana (*Musa* spp.) Cultivars from Excised Shoot Tips  
**S. Shirani, M. Maziah, M. Sariah, W.B. Zakaria and F. Mahdavi** ........................................109

The Effect of Ethyl Methane Sulphomate (EMS) and Sodium Azide (NaN₃) on Plant Regeneration Capacity of Banana Cultivar ‘Yueyoukang 1’ (AAA), Highly Resistant to Fusarium Wilt  
**C.X. Xu, J. Xiao, J.G. He, G.B. Hu and H.B. Chen** ..............................................................110

State of the Culture of the Banana Tree in a Mediterranean Zone: The Tunisian Coastal Oases of Gabes  
**M. Ben Salah, A. Ferchichi and M. Jeridi** ........................................................................111

Evaluation of Some Cavendish Cultivars (*Musa* spp., AAA) under Plastic Greenhouses in Subtropical Areas of Turkey  
**H. Gubbuk, F. Bakry and Y. Mathieu** ...............................................................................112
Session 2: Integrated Approaches to Managing Fusarium Wilt and Other Emerging Disease Threats

Monitoring Fusarium Wilt Race 4 in Hainan Province and Populations of *Fusarium oxysporum* f. sp. *cubense*
X. Zhang, H. Zhang, Y. Xie, J. Pu, Y. Qi and Y. Lu ................................................................. 113

Risk Analysis on Introduction of Banana Fusarium wilt to Yunnan
L. Zeng, X.D. Li, H.C. Fan and Z.X. Guo ........................................................................... 114

Status of Fusarium Wilt in India
H.P. Singh ................................................................................................................................. 114

First Report on the Occurrence of a Virulent Strain of *Fusarium oxysporum* f. sp. *cubense* VCG 0124 (Race 1) Infecting Cavendish (AAA) Banana
R. Thangavelu, M. Gopi and M.M. Mustaffa ............................................................................. 115

Banana Cultivars and *Fusarium oxysporum* f. sp. *cubense* in Indonesia – Observations from Fusarium Wilt Disease Databases
J.W. Daniells, W. O’Neill, C. Hermanto and R.C. Ploetz .......................................................... 116

A Molecular Detection Method Specific to *Fusarium oxysporum* f. sp. *cubense* Race 4 in Taiwan
P.F.L. Chang, Y.H. Lin, J.Y. Chang, C.P. Chao and J.W. Huang ................................................. 117

Cloning and Diversity Analysis of FGA1 from Two *Fusarium oxysporum* formae speciales
C.Y. Li, G.J. Yi, S. Chen, Q.M. Sun, C.W. Zuo, B.Z. Huang, Y.R. Wei, Y.H. Huang, Y.L. Wu, L.B. Xu and C.H. Hu ......................................................................................................................... 118

Comparison of Three Inoculation Techniques for Pathogenicity Tests on Fusarium wilt of Banana
C.E. Soguilon, L.E. Herradura, A.G. Yebes, V.O. Sinohin and A.B. Molina ............................. 118

Reporter Gene-Labelled *Mycosphaerella fijiensis* and *Fusarium oxysporum* f. sp. *cubense* as Tools for Pathogenicity Studies

Correlation between Susceptibility for Pathogen and Crude Toxin of *Fusarium oxysporum* f. sp. *cubense* race 4 in Banana Varieties Plantlets
X.J. Yang, Y.X. Du, F.R. Chen, L. Gan and H.C. Ruan .............................................................. 120

Field Evaluation of Banana Genotypes for Resistance to Fusarium Wilt
S.O. Silva, L.R. Ribeiro, E.P. Amorim, Z.C. Cordeiro, M.C. Lima and M.A. Dita .......................... 121

Reaction of *Musa* Hybrids to Fusarium wilt and Burrowing Nematode Complex
T.N. Balamohan, Suken Chandra Das, K. Poornima and N. Seenivasan ................................. 122
Table of Contents

Development of In-Vitro Rooted Banana Plantlets-
*Fusarium oxysporum f. sp. cubense* Interaction System in Erlenmeyer Flasks

Isolation and Characterisation of Endophytic Bacteria from Fusarium Wilt-Resistant Banana Plants and Evaluation of their Antipathogenic Activities against *Fusarium oxysporum f. sp. cubense*
R. Thangavelu, P. Ganga Devi, M. Gopi and R. Baby Shalini ................................. 124

Acidifying Amendments and Fusarium Wilt Incidence in Banana in Indian Peninsula
M. Edward Raja and P.N. Krishnamurthy ................................................................. 125

Evaluation of host reaction of *Musa* germplasm to the Banana Corm Weevil, *Cosmopolites sordidus*
B. Padmanaban, S. Uma and M.M. Mustaffa ....................................................... 126

Evaluation of Seeded Banana for Biotic Stresses

Screening of Banana Germplasm for Resistance/Tolerance to *Pratylenchus coffeae*
P. Sundararajju, T. Sekar, S. Uma, P. Saravanan and M.M. Mustaffa ........................ 128

Banana Nematode Control using Argan and Other Medicinal Plants
Z. Ferji, H. Mayad, L. Bouhaddou and D. De Waele ............................................. 129

Screening of Banana Varieties/Germplasm against Foliar Diseases
A.N. Sabalpara, Priya John, K.U. Solanky and B.P. Mehta ........................................ 130

The Importance of Black Leaf Streak Control in ‘Pisang Berangan’ (AAA) in Peninsular Malaysia
K.H. Then and S. Palaniappan .................................................................................. 131

Isolation and Characterisation of Endophytic and Epiphytic Bacteria from Sigatoka Leaf Spot-Resistant Plants and Evaluation for their Antipathogenic Activities against *Mycosphaerella* spp.
R. Thangavelu, R. Baby Shalini, M. Gopi and P. Ganga Devi ................................. 131

Community Coping Mechanisms in Response to Xanthomonas Wilt Epidemics in Uganda
E. Karamura, L. Aliguma and W. Tinzaara ............................................................... 132

The Drivers of Banana Xanthomonas Wilt Epidemic in East and Central Africa
W. Tinzaara and E.B. Karamura .............................................................................. 133

Bacterial Rhizome Rot – A New Threat to French Plantain Cultivar ‘Nendran’ (*Musa*, AAB) in India
A. Cherian, N.K. Usha and R. Menon ........................................................................ 134

Current Status, Diagnosis and Management of Viral Diseases of Banana in Kerala, South India
A. Cherian, R. Menon, P.M. Mathew, A. Suma and K.C. Aipe .................................... 135
Table of Contents

Symptomless Banana Plants and Other Species as Reservoir of BBTV Inoculum
F. dela Cueva, E. Dinglasan, F.S. dela Cruz, V. Sinohin and A.B. Molina........................................ 136

CR-M: An Important Nucleotide Acid Sequence to Distinguish BBTV Isolates from Two Groups
Y.H. Huang, J.W. Zeng, Y.L, Wu, R. Xia and G.J. Yi ........................................................................ 137

Towards Transgenic Resistance to Banana Bunchy Top Virus (BBTV) by Expression of Defective Viral Reps
A.M. Njoroge, R.J. Geijskes, R.M. Harding, A.P. James, T.T. Tsao, D.K. Becker
and J.L. Dale ................................................................................................................................. 138

Banana Tissue Culture for Production of Banana Bunchy Top Virus (BBTV)-Free Plants in Pakistan
A. Muhammad, H. Rashid, I. Hussain, S. Masood and S.M. Saqlan Naqvi ........................................ 139

Establishment of Banana Virus-Indexing Centres and Surveying for Banana Viruses in East Africa
A.P. James, J. Mugini, C. Changa, J. Kubiriba, L. Karanja, R.J. Geijskes, R.M. Harding
and J.L. Dale .................................................................................................................................. 140

Novel Approaches for Identifying Nematode Problems in Tissue-Culture Banana in India
P. Sundararaju and M.M. Mustaffa .................................................................................................... 141

Investigation into Low-Cost Medium for Hardening of In-Vitro Banana Plantlets
B. Jhurree-Dussoruth and H. Kallydin ............................................................................................... 142

Performance of Banana Cultivars (Musa spp.) Propagated by Tissue Culture and Suckers in India
M.H. Dahale .................................................................................................................................. 143

Micropropagation of 'Pisang Awak' (Musa, ABB genome) as a Model for Enhancing and Improving Livelihood of Rural Communities in Malaysia
L.K. Chan, V.H. Au, A.A.A. Noor, M. Adnan and P.L. Boey .............................................................. 143

Effect of Soil Moisture Deficit Stress on Physiological and Biochemical Parameters of Banana Plants
I. Ravi, M.M. Mustaffa and M. Mayilvaganan ................................................................................. 144
Oral Presentations
Opening Session

Opening Keynote 1: Status, Challenges and Trends of the Chinese Banana Industry

G.J. Yi, C.Y. Li, Y.L. Wu, B.Z. Huang and Y.R. Wei

Fruit Tree Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou, 510640, P.R. China

Banana is one of the most important fruit crops in China with a production of 7,050,000 tonnes on an area of 279,500 hectares in 2008. China is thus the third biggest producer in the world. Yet, China imported about 365,000 tonnes of banana in 2008. Most of the bananas are sold in the domestic market. As the demand for banana in the country is very high, banana produced domestically can only meet 90% of the demand, the other 10% is met by imported banana. Banana is mainly cultivated in Guangdong, Guangxi, Hainan, Fujian, Yunnan Province and Taiwan. Guangdong’s cultivated area and production quantity rank first in China. The main banana cultivars in China belong to the Cavendish subgroup (AAA) (99.1%), including the cultivars ‘Baxi Jiao’, ‘Williams (8818)’, ‘B6’, ‘Guangdong Banana No.2’, ‘Aijiaodundilei’, ‘GaoJiaodundilei’, ‘Tianbaogao Jiao’, ‘Fenjiao’ and ‘Dajiao’. Cultivars of the AAB and ABB genomic group represent 5.3%, and ‘Longyajiao’ (AA group) accounts for 3.7%. The banana industry in China has been evolving fast over the past two decades. Indeed, banana production is a crucial industry in each main producing area; it plays an important role in the local economy and rural development. Many high-yielding and good-quality production techniques have been widely adopted by growers, such as micropropagated plantlets, water-saving irrigation, fertilisation, bunch management techniques, etc. However, Fusarium wilt and adverse weather conditions, including typhoons and low temperature, are threatening the production. More than 3,000 hectares of banana plantations have been attacked by Fusarium wilt in Guangdong.
Production declined by more than 30% due to cold temperature in 1991-1992, 1999-2000 and 2002-2003, and the damage in 2007-2008 was devastating. In order to promote a healthy and persistent development of the banana industry, the National Industry System of Banana was initiated by the National Agricultural Ministry in 2008. Scientists were organised to tackle key issues, including breeding, cultivation technology, postharvest and processing, marketing and trade.

**Opening Keynote 2: Using *Musa* Genes in the Fight against Production Constraints**

E. Frison

*Bioversity International, Via dei Tre Denari 472/a, 00057 Maccarese (Fiumicino) Rome, Italy*

Genetic diversity of a crop can be utilised, either directly as a mixture of different cultivars grown in the same field or indirectly in crop improvement programmes, to increase yield or to maintain productivity under conditions of stress. Like other crops, banana and plantain (*Musa* spp.) are threatened by many abiotic and biotic stresses. In Asia, the *Banana bunchy top virus* and a new strain of the *Fusarium wilt* pathogen are only two examples of the devastating effect such diseases can have on the banana industry, and thus on people’s livelihoods. Fortunately, Asia is also home to a wide range of wild and cultivated varieties, which can be exploited to make banana production more resilient and sustainable. The author will present some examples of Bioversity research experiences and success stories to draw lessons about how *Musa* genetic resources can be used more effectively for sustainable production systems, better nutrition and improved livelihoods for the poor.
Keynote 1: Non-Conventional Breeding of Banana (Musa spp.)

X.L. Huang, X. Huang, W. Xiao, J.T. Zhao, X.M. Dai, Y.F. Chen and Q. Gong

Key Laboratory of Gene Engineering of the Ministry of Education, School of Life Sciences, Zhongshan (Sun Yat-Sen) University, Guangzhou 510275, China

Biotechnology, and more specifically genetic transformation, protoplast culture and somatic hybridisation, support alternative methods in banana improvement. This paper presents some research in non-conventional breeding of banana carried out at the Key Laboratory of Gene Engineering, China. By using explants of immature flowers, embryogenic cell suspensions (ECS) were established of several banana cultivars, including ‘Pisang Mas’ (AA), ‘Guo Shan Xiang’ (AAB, Silk), ‘Ba Xi Jiao’ (AAA, Cavendish) and ‘Dong Guan Da Jiao’ (ABB), which is a local banana cultivar resistant to Fusarium oxysporum f. sp. cubense (Foc) race 4. The results show that pre-culturing the ECS of ‘Dong Guan Da Jiao’ and ‘Ba Xi Jiao’ in M2 medium without 2,4-D for 10 days enhanced the frequency of somatic embryogenesis 1.7-fold. To attempt to introduce genetic information of disease resistance from ‘Dong Guan Da Jiao’ to ‘Guo Shan Xiang’ and obtain somatic hybrids, an asymmetric protoplast fusion with polyethylene glycol was developed. A total of 47 regenerated green plants were obtained, eight of which survived in the greenhouse. Six of the surviving plants were identified as hybrids by RAPD-ISSR (Random Amplified Polymorphism DNA - inter-simple sequence repeat) and GISH (genomic in situ hybridisation) analysis. We also studied the asymmetric somatic hybridisation of ‘Ba Xi Jiao’ and ‘Dong Guan Da Jiao’. Cell colonies were formed from the fusion products after 45 days on a feeder layer culture, and 1236 somatic embryos were obtained. However, they all failed to germinate in further
culture. We cloned a novel full-length NPR1-like gene, designated MdNPR1 (accession number FJ357442) from ‘Dong Guan Da Jiao’. The transcripts of MdNPR1 accumulated stronger in ‘Dong Guan Da Jiao’ than in ‘Fen Jiao’ (AAB), a local cultivar which is very sensitive to Foc race 4. MdNPR1 were also constructed and transferred into ECSs of ‘Ba Xi Jiao’ via Agrobacterium-mediated method. The expression of GUS gene could be detected in the transformed ECS after co-culture and in the putative transformed somatic embryogenesis, but regeneration was not achieved.

Keynote 2: From Fundamental Research Discoveries to Applications for Banana Improvement


Laboratory of Tropical Crop Improvement, Katholieke Universiteit Leuven (K.U.Leuven), Kasteelpark Arenberg 13 bus 2455, 3001 Leuven, Belgium

An overview of the challenges, opportunities, recent achievements and future research needs in the area of banana biotech improvement will be presented using current research at the Catholic University of Leuven (K.U.Leuven), Belgium as a springboard. The Global Banana Collection at the Musa International Transit Centre (ITC) maintains nearly 1200 banana (Musa spp.) accessions in vitro. Through the Global Conservation Strategy, ITC is linked to field collections, which are used for taxonomy training and data acquisition on – for example – resistance towards black leaf streak, Fusarium wilt and nematodes. Due to unstable genomic regions and epigenetic modifications, banana accessions exhibit somaclonal variation. For some accessions, the frequency of somaclonal variation increases dramatically under in-vitro conditions. In order to safely store this valuable Musa biodiversity, the in-vitro collection is being cryopreserved. Over the years, fundamental research has proved instrumental in the successful development of the different cryopreservation protocols. Among others, a proteomics study was performed on meristem cultures to investigate the response of different banana cultivars to osmotic stress associated with cryopreservation. Valuable proteomics experience in Musa and other crops was gained based on two-dimensional gel electrophoresis (2DE). Using high-resolution 2DE gels that routinely display
about 900-1500 protein spots, individual protein isoforms were separated and an extensive amount of mass spectrometry data accumulated. Ultimately, a 2DE map of the *Musa* meristem proteome (637 identified proteins) was constructed. Following identification, some of the differentially expressed proteins under osmotic stress were further functionally characterised in engineered plants. This banana transformation platform is also used as a tool for gene and promoter discovery via T-DNA tagging. Other groups have transformed banana for production of engineered banana plants for resistance to biotic stress or increased provitamin A content. In these cases, foreign genes were used or cloned from banana based on homologues sequences in other crops. These biotechnological approaches constitute a significant step towards the development of better performing crops, including more stress-tolerant varieties of banana.

**Genomics, Banana Breeding and Superdomestication**

J.S. Heslop-Harrison

*University of Leicester, Leicester LE1 7RH, UK*

Superdomestication involves a partnership of breeders and genomic scientists to design the suite of characteristics required from an ideal banana cultivar. There is then the need to find and evaluate the genes responsible for the characters, ranging from biotic and abiotic stress resistance, through yield, to post-harvest ripening and storage qualities. Some involve single genes but others are quantitative trait loci with many genes involved and with changing expression patterns depending on conditions. The genes within the diverse accessions in *Musa* germplasm collections underpin the search for desirable traits, now requiring work targeted to finding, measuring and conserving the biodiversity. Many genomic resources have been made available for *Musa* through the Global *Musa* Genomics Consortium, including the DNA sequences and DNA libraries, along with extended plant collections, hybrid and mapping populations. We can also exploit parallels with other crops to identify gene functions and similarities in genetic structure. Now, this information can be used to evaluate the applicability and diversity of germplasm and its precise properties, characterise stress-related and other genes and to measure and map quantitative trait loci. After a superdomesticate has been designed, the
approaches to produce it can be considered, whether resynthesising hybrids from desirable wild relatives or hybridising elite material with residual fertility, or transgenic approaches. The breeder will generate the elite plants, but the known value of the outcome, and the possibility of using DNA based tools to assist the selection, can make a desirable elite variety much more likely to be found. Expertise from breeders and farmers, complemented with exploitation of the genepool and use of genomic sciences for characterisation and breeding, opens up a new range of opportunities for the future of the banana crop, although undoubtedly requires further development of *Musa* genomics. Further information and references are available from www.biobanana.com.

Analysis of Genome Structure and Organisation in Banana (*Musa acuminata*) Using 454 Sequencing

E. Hřibová<sup>1</sup>, P. Neumann<sup>2</sup>, J. Macas<sup>2</sup> and J. Doležel<sup>1</sup>

<sup>1</sup>Laboratory of Molecular Cytogenetics and Cytometry, Institute of Experimental Botany, Olomouc, Czech Republic; <sup>2</sup>Biology Centre ASCR, Institute of Plant Molecular Biology, Laboratory of Molecular Cytogenetics, České Budějovice, Czech Republic

Diploid and polyploid forms of *Musa acuminata* (A genome) and hybrids that originated from crosses between *M. acuminata* and *M. balbisiana* (B genome) represent most of the edible banana cultivars. Although the genomes of both species are relatively small (1C ~ 550-650 Mbp), the knowledge on genome organisation and evolution remains poor. The availability of the next generation sequencing technologies, which produce large numbers of sequences in a single run, provides an opportunity to fill the gap. In this work, we used the massively parallel 454 sequencing technology to characterise repetitive DNA sequences in *M. acuminata* ssp. *burmannicoides* ‘Calcutta 4’ (1C ~ 620 Mbp). One 454 sequencing reaction on Roche GS-FLX system resulted in 477,699 reads with average length of 206 nucleotides, providing a total of 100 Mb sequence. Until now, this is the largest amount of genomic sequence data available for *Musa*, representing about 15% of the ‘Calcutta 4’ genome. The 454 reads were assembled into contigs and various types of mobile elements, and new tandem organised repeats were classified and characterised for copy number and genomic distribution. Localisation of new tandem repeats using FISH on mitotic chromosomes revealed their clustering in a subtelomeric region of one
chromosome pair. Moreover, we studied genomic organisation of the new tandem repeats in relatives of *M. acuminata*. We envisage that the 454 sequence data obtained in this work will facilitate annotation of nucleotide sequences during the ongoing banana genome sequencing project and will be a useful resource for developing new DNA markers. This work was supported by the Grant Agency of the Academy of Sciences of the Czech Republic (grant award no. KJB 500380901) and the International Atomic Energy Agency (research agreement no. 13192).

**Genome-Wide BAC End Sequencing of *Musa acuminata* ‘DH Pahang’ Reveals Further Insights into the Genome Organisation of Banana**

M.T. Souza Jr\(^1\), N. Roux\(^2\) and G.H.J. Kema\(^3\)

\(^1\)Embrapa LABEX Europe, 6708 PB, Wageningen, The Netherlands; \(^2\) Bioversity International, Parc Scientifique Agropolis II, 34397 Montpellier Cedex 5, France; \(^3\)Plant Research International, 6708 PB, Wageningen, The Netherlands

A *Musa acuminata* ssp. *malaccencis* ‘DH Pahang’ BAC library, named MAMB, was used for BAC end sequencing. MAMB was constructed by Amplicon express (WA, USA) using DNA from field-grown cigar leaves, by cutting with BamH1, and cloning in pCC1BAC (EPICENTRE) / *E. coli* DH 10B. This DH Pahang Library consists of 23,040 clones, with a 140 kbp average insert size, accounting for a 5X coverage of the banana A genome. The BES project of MAMB was developed under the scope of the strategic alliance between Embrapa (Brazilian Corporation for Agriculture Research) and Wageningen University in the MusaForever and PRPB (Pesticide Reduction Programme for Banana) initiatives. Sequencing was performed at the HudsonAlpha Institute for Biotechnology (Al, USA), and a total of 46,080 reads was generated. After trimming for vector and quality, these reads generated 42,750 high-quality sequences, with a Phred average of 661.7 bp, and comprising 30.5 Mb of Phred 20 sequences. This database of BAC end sequences is essential for the assembly of the complete banana genome sequence, a recently started collaborative programme between Génoscope, CIRAD, Embrapa and Wageningen University funded by the French National Research Agency (ANR).
Identification and Validation of EST-Derived Molecular Markers, TRAP and VNTR, for Banana Research

S.A.L. Garcia\textsuperscript{1,4}, R. Talebi\textsuperscript{1}, C.F. Ferreira\textsuperscript{2}, I. Vroh\textsuperscript{3}, L.V. Paiva\textsuperscript{4}, M.T. Souza Jr\textsuperscript{1,5} and G.H.J. Kema\textsuperscript{1}

\textsuperscript{1}Plant Research International, 6708 PB, Wageningen, The Netherlands;  \textsuperscript{2}Embrapa Cassava & Tropical Fruits, Cruz das Almas, 44380-000, Bahia, Brazil;  \textsuperscript{3}International Institute of Tropical Agriculture (IITA) - Nigeria Ibadan, PMB 5320, Ibadan, Oyo State, Nigeria;  \textsuperscript{4}Universidade Federal de Lavras, Caixa Postal 3037, Lavras- MG, Brazil;  \textsuperscript{5}Embrapa LABEX Europe, 6708 PB, Wageningen, The Netherlands

The advent of high-throughput sequencing technology has generated abundant information on DNA sequences for the genomes of many plant species. Expressed Sequence Tag (EST), a unique DNA sequence derived from a cDNA library and therefore representing a gene which has been transcribed in a specific tissue or at some stage of development, is one type of DNA sequence made highly available lately for many important crop species. Molecular markers are used for bridging DNA sequence information with particular phenotypes and are useful tools for genotyping germplasm collections and also for tagging genes governing desirable agronomic traits. In this sense, there is always a strong demand for better marker techniques to better utilise the existing sequence information. A transcriptome database from banana (\textit{Musa} spp.), DATAMusa, containing 42,724 ESTs from 11 different cDNA libraries and encompassing approximately 24 Mb of DNA sequence, was used in this study for the design of primers to PCR amplify two types of EST-derived molecular markers, Variable Nucleotide Tandem Repeat (VNTR) and Target Region Amplification Polymorphism (TRAP). These primers were then validated against a panel of 14 \textit{Musa} diploid genotypes and produced 33 (VNTR) and 119 (TRAP) alleles. Used separately or together, both types of markers were able to discriminate \textit{Musa} genotypes from different genome background (A or B genomes). The TRAP alleles identified derived from only one unigene, while the VNTR derived from 12 unigenes. Based on the results from this study, it can be said that EST-derived markers are an important source of polymorphism to be used in genetic diversity and gene discovery studies in banana.
Wild *Musa* species in China

M. Häkkinen

*Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun, Mengla, Yunnan Province 666303, People’s Republic of China; and Botanic Garden, PO Box 44 (Jyrängöntie 2), FI-00014, University of Helsinki, Finland*

Several investigations of Musaceae plants in southern China over the past 50 years—including extensive field trips conducted by the author in Guangdong, Guangxi, Yunnan and Hainan between 2005 and 2007—have shown that most of the wild bananas (*Musa* spp.) are located in areas where there is no agriculture: on mountain slopes as high as 2,250 meters and in nature reserves. In those areas, the climate varies substantially. At lower elevations, the climate is tropical to subtropical, suitable for year-round flowering. At higher elevations, especially in the northern parts of Yunnan, Guangxi and Guangdong, the climate generally restricts flowering to the period from July to September. In December and January, frost damage can occur. The ecology of some of these species suggests that they possess characteristics which could be of interest to breeders. For example, a newly described species, *Musa yunnanensis*, is found in cold locations where it withstands subzero temperatures, a trait that could be used to breed for tolerance to cold. For other species, the interest may lie in their resistance to diseases. For example, another newly described taxon, *Musa acuminata* var. *chinensis*, is closely related to *M. acuminata* ssp. *burmannicoides* ‘Calcutta 4’, which has been extensively used in breeding because of its resistance to black leaf streak. These newly described taxa have in common monoclinous hermaphroditic female flowers that self-pollinate before the bract opens. Consequently, no hybrid of these two taxa has ever been observed. The author also observed ten species that belong to the Musa section, one to the Callimusa section and four to the Rhodochlamys section, in addition to two species of the genus *Ensete* and one species of the genus *Musella*. Field evaluations are needed to identify the wild species that could be used in breeding programmes.
**Musa Collection and Characterisation in Central and Eastern DR-Congo: a Chronological Overview**

B. Dheda Djailo¹, B.D. Nzawele², N. Roux³, F. Ngezahayo⁴, N. Vigheri⁵, E. De Langhe⁶, D. Karamura³, S. Channelière³, M. Ruas³, C. Picq³ and G. Blomme³

¹University of Kisangani (UNIKIS), Kisangani, DR-Congo; ²INERA, Mulungu, DR-Congo; ³Bioversity International, Parc Scientifique Agropolis II, 34397 Montpellier Cedex 5, France; ⁴Institut de recherche agronomique et zootechnique (IRAZ), Gitega, Burundi; ⁵UCG, Butembo, DR-Congo; ⁶Catholic University of Leuven (K.U.Leuven), Leuven, Belgium

*Musa* collection and characterisation has been ongoing in DR-Congo since the mid 1950s. Initial efforts were made by the *Institut national des études agronomiques au Congo* (INEAC). Plantains (AAB) were mainly collected in Oriental Province, while East African highland bananas (AAA) were collected in South Kivu. The collections established at the INEAC Yangambi research station and at Mulungu, South Kivu no longer exist due to social unrest and instability in the region. However, the study of these collections revealed that humid Africa is the major secondary diversity centre for both *Musa* groups. In the framework of the *Communauté économique des pays des Grands Lacs* (CEPGL), collecting missions were carried out from 1984 till 1991 in South and North Kivu. Collected accessions were established at the *Institut de recherche agronomique et zootechnique* (IRAZ) in Gitega, Burundi. Only 32 out of the 67 genotypes collected in eastern DR-Congo are currently still present at the IRAZ collection. However, all these initial missions only collected a fraction of the numerous plantain and highland banana genotypes present in DR-Congo. From 2005 till 2007, three collecting missions were carried out by the University of Kisangani (UNIKIS) to collect plantains in Oriental Province and to recover a great deal of the extinct plantain collection of Yangambi. Sixty-five plantains were collected and established in a field collection at UNIKIS, and characterisation work is ongoing. These plantains comprise some interesting dwarf and semi-dwarf varieties which are not present in West Africa. In 2006, a PhD study was started in the framework of the Consortium for Improving Agriculture-based Livelihoods in Central Africa (CIALCA) project to collect and characterise all the available *Musa* germplasm in South Kivu, North Kivu and in the eastern part of Oriental Province of DR-
Congo. So far, 196 *Musa* genotypes have been collected, and morphotaxonomic characterisation is ongoing. Synonyms need to be identified and ploidy analysis is planned. The 196 cultivars were established in three *Musa* collections (two in North Kivu and one in South Kivu). Information on all the collected genotypes in DR-Congo will be entered in the *Musa* Germplasm Information System (MGIS). Many new accessions represent plantains adapted to higher altitudes which have hardly been explored hitherto. These plantains should be ecotypes of which the genetic study could strengthen breeding efficiency. Many plantains are unique to the African continent and could be introduced into Asia for specific characteristics (e.g. in South India, where the rather drought-tolerant False Horn plantains are completely missing).

**Morphological Characterisation of East African AAB and AA Dessert Bananas (*Musa* spp.)**

M. Onyango¹, D. Karamura², S. Keeley³, R. Manshardt⁴ and D. Haymer⁵

¹Kenya Agricultural Research Institute (KARI) - Kisii, PO Box 523, Kisii, Kenya; ²Bioversity International - Uganda, PO Box 24384, Plot 106, Katalima Road, Naguru, Kampala, Uganda; ³University of Hawai’i at Manoa, Department of Botany, 3190 Maile Way 101, HI 96822, Honolulu, USA; ⁴University of Hawai’i at Manoa, Department of Tropical Plant and soil Science, 3190 Maile Way 102, HI 96822, Honolulu, USA; ⁵University of Hawai’i at Manoa, Department of Cell and Molecular Biology, 1960 East West Road T511, HI 96822, Honolulu, USA

Among the small-fruited AAB dessert bananas in East Africa are cultivars that have potential for export, and the diploid AA bananas known in Kenya as “Muraru” are socially valued in the country. It is important to be able to distinguish these cultivars from other similar cultivars and identify the various subgroups within the AAB and AA genome groups, and possibly come up with some recommendations for future economical considerations. Objectives of this study were 1) to identify morphological characters that distinguish a) the various subgroups of AAB dessert bananas found in East Africa, and b) the Muraru from other cultivated AA bananas; 2) to evaluate the relationship among the AAB and among the AA Muraru dessert banana groups of East Africa in relation to other bananas. Forty-three cultivars of AAB, AA groups and outgroups from a large banana collection at Kenya Agricultural Research
Institute, Kisii were characterised in 2007 using morphological traits. Morphological data were collected using 84 characters derived from a modified version of the descriptors for bananas developed by Bioversity International in conjunction with CIRAD. Techniques of multivariate analysis were employed. Based on unweighted pair group using arithmetic mean (UPGMA), two major clusters of *Musa acuminata*-derived cultivars (AAs and AAAs) and hybrids of *Musa balbisiana* and *M. acuminata* (AAB) were produced. Within the major clusters were subclusters conforming to various subgroups. Within the AAB dessert cluster, four distinct subclusters were formed, i.e. Sukari Ndizi, Prata, Mysore and Silk. Muraru also formed a well-defined cluster. Thirty-three (33) characters contributed 71% of the total variation within the 43 accessions on the first and second principal components, allowing separation of clusters corresponding to genome groups and subgroups. The analysis further revealed that morphological traits, particularly of male bud, fruit and sucker, can be used to make distinctions within genome groups and subgroups and to be able to isolate various subgroups within their genome groups. Morphological traits can be used confidently to describe various banana subgroups.

The Complementarity of Farmer’s’ and Botanical Descriptors of the East African Highland Banana Cultivars (*Musa*, AAA)

D. Karamura¹, A. Kiggundu² and E. Karamura¹

¹Bioversity International, PO Box 24384, Kampala, Uganda; ²National Agricultural Research Laboratories Institute, Kawanda Banana Programme, PO Box 7065, Kampala, Uganda

Farmers in East Africa help shape the degree of genetic diversity in banana landraces. They describe cultivars by names related to one or more traits at various development stages of the plant life cycle, like agronomic performance, uses of plant parts or aesthetics. This allows farmers to categorise diversity using relevant morphological criteria to create a pattern of naming and grouping cultivars. Factors farmers use to describe and name cultivars are interrelated and provide a set of agromorphological criteria which define a landrace. Not much is known about the structure of farmers’ nomenclature of these landraces and its relevance to the botanical descriptors and classification. This paper describes a study which was undertaken in two culturally different
banana-growing communities in Uganda: Luwero in mid-altitudes and Mbale in high-altitudes. The purpose of the study was to identify the traits farmers use in describing and naming cultivars of East African highland banana, to compare farmers’ and botanical descriptors to assess the relationship between the two and to determine the biological usefulness of the farmers’ system. Three methods were used: an informal participatory method to provide preliminary information on descriptors; a quantitative ranking of the results of the participatory method; and multivariate statistics to discover the relationship between the two types of descriptors by comparing the categorisations resulting from the descriptor analysis. Results indicated that the most important farmer descriptors fell into five categories: size and shape, texture, appearance, agronomic aspects and commercial aspects. There was more than 60% correlation between farmers’ grouping of cultivars (based on fewer descriptors) and botanical classification (based on many descriptors). In conclusion, farmers’ grouping resulting from their descriptors had a biological meaning while the botanical classification reflected the practices of local people.

Standardised Procedures for *Musa* Germplasm Characterisation – Use of a Reference Collection – Towards a *Musa* Identification Key?

S. Channelière¹, A. Vezina¹, E. Arnaud¹, J-P. Horry², M. Ruas¹ and N. Roux¹

¹Bioversity International, Parc Scientifique Agropolis II, 34397 Montpellier Cedex 5, France; ²Centre de coopération internationale en recherche agronomique pour le développement (CIRAD), Montpellier, France

The genetic diversity of *Musa* germplasm is a very important asset to support efforts to increase banana productivity, making the crop more resistant to pests and diseases and safeguard it against environmental changes. Bioversity, managing the world’s largest *Musa* germplasm collection at the International Transit Centre (ITC), is playing a major role in the global conservation effort of *Musa* and in the exchange and use of germplasm. Efficient germplasm characterisation is a prerequisite, not only to facilitate the use of germplasm diversity, but also to prioritise resources towards accessions of interest and not yet conserved. The *Musa* Taxonomy Advisory Group (TAG) identified the lack of a comprehensive procedure to classify an accession up to subgroup and
subspecies level as a bottleneck for efficient *Musa* conservation. The TAG decided to define a ‘Reference Collection’, representing the major taxonomic subgroups of edible triploids and diploids and a sample of wild species. The Reference Collection comprises 35 accessions freely available for distribution. The accessions can be used for research purposes, to support classification of materials in genebanks and for training on taxonomy in national germplasm collections. A preliminary list of 31 minimum descriptors and a set of 15 photos were agreed on as a first tool for characterisation. The 35 accessions of the Reference Collection will be sent to several collections, to allow us i) to improve and standardise a procedure for characterisation using molecular techniques, morphological descriptors and sets of photos, ii) to test the robustness of descriptors across different environments, iii) to develop tools to allocate an accession to a subgroup or a subspecies (scoring system, identification keys), and iv) to have a limited set of accessions serving as a model of each subgroup for research, classification and training. Results of the characterisation will be made available via the online *Musa* Germplasm Information System (MGIS).

**Diversity Analysis in Indian Cooking Bananas (ABB) through Morphotaxonomic and Molecular Characterisation**

M.S. Saraswathi, S. Uma, E. Vadivel, P. Durai, S.A. Siva, G. Rajagopal and S. Sathiamoorthy

*National Research Centre for Banana (NRCB-ICAR), Thogamalai Road, Thayanur (Post), Tiruchirapalli 620102, Tamil Nadu, India*

Improvement of ABB cooking bananas and ‘Pisang Awak’ (ABB) is one of the breeding objectives of the National Research Centre for Banana (NRCB), India. India is one of the main centres of origin and domestication of *Musa balbisiana*. The ability of the species to introgress naturally with *Musa acuminata* has contributed to the vast diversity of B and B-rich genomes in India. The NRCB field genebank has a large collection of 125 cooking banana accessions, dominated by Monthan and Bluggoe subgroups. Phylogenetic studies could unveil the diversity available in the cooking bananas for potential exploitation in genetic improvement programmes. In the present study, the ABB germplasm was characterised through morphotaxonomic traits and
molecular markers, including AA and BB genomes as reference groups. The
data were subjected to Hierarchical Cluster Analysis (HCA) and Principal
Component Analysis (PCA) using NTSYS. Out of 125 accessions characterised
morphotaxonomically, only 71 accessions were distinct and the rest (54) were
synonyms. Forty-eight accessions which were suspected to be synonyms based
on morphotaxonomic characterisation were characterised using 36 pairs of
microsatellite markers. In both the systems, cluster analysis resulted in two
major clusters. Cluster 1 comprised AA accessions and cluster 2 included BB
and ABB accessions. Though Monthan and Bluggoe are the major subgroups,
the present characterisation has resulted in another subgroup called Bontha,
which has clustered along with Montan sharing only 40% similarities. The
cophenetic correlation coefficients estimated were significant (0.98) in both
molecular and morphotaxonomic characterisation, indicating a good fit of the
dendrogram with the similarity matrices produced. The two-dimensional scatter
plot obtained as a result of PCA also confirmed the clustering patterns
elucidated by HCA. The phylogenetic relationships derived in the present
study, complemented with fertility studies, helped in the identification of
diverse parents which could be used for the development of superior hybrids.
This also facilitated the elimination of synonyms during the establishment of a
comprehensive core collection at NRCB.

**Application of Cytoplasmic and Nuclear DNA-Based Marker
Systems for Elucidation of the Phylogenetic Relationship of
*Musa acuminata* and *Musa balbisiana* and their Hybrids**

R. Boonruangrod, D. Desai, M. Berenyi, S. Fluch and K. Burg

*Austrian Research Center GmbH–ARC, Department of Biogenetics, A-2444 Seibersdorf, Austria*

*Musa* (Musaceae) is one of the most important staple crops in the tropics and
subtropics. The present-day edible banana cultivars originate mostly from the
diploid species (2n= 22) *Musa acuminata* and *M. balbisiana*. The diploid or
polyploid banana cultivars are sterile intra- or inter-specific hybrids of these
two species and have been fixed through hundreds/thousands of years of human
selection. The genetic improvement of banana is slow because knowledge on
the putative fertile ancestors is scanty. The present study was based on the assumption that the ancestors of the present day wild types were participating in the ancient hybrid formation and these ancient genomes are still analysable in the present-day cultivars because of their limited possibility for recombination in the mostly sterile hybrids. Therefore, uniparentally inherited cytoplasmic (maternally inherited chloroplast and paternally inherited mitochondria) as well as biparentally inherited nuclear genome-based marker system (rDNA) were applied for the identification of putative ancestor genepools of banana. This study was based on a mini core collection of 52 genotypes, including ten *M. acuminata* and eight *M. balbisiana* wild types along with ten AAA, ten AAB, eight ABB triploid, three AA, two AB diploid and a single tetraploid cultivar. Genepools featured by present-day wild types could be established with all three marker systems for *M. acuminata*, while *M. balbisiana* yielded no variability for the rDNA locus. Using the cytoplasmic and nuclear marker systems separately, genepools representing the ancient genomes featured by present-day wild types have been established. Using the genepool classification for tracing the maternal lineage by chloroplast and the paternal lineage by mitochondrial genepools in combination with nuclear rDNA genepools allowed the identification of putative family trees, suggesting simple ways for the evolution of the present-day hybrid cultivars. In addition, our results indicate that certain wild genotypes are lacking in collections, strengthening the need for more but clearly focused collecting missions.

**Phylogenetic Relationships in the Family Musaceae Based on the Genic Sequences, Sequence of the ITS1-5.8S-ITS2 Region and DArT Markers**

P. Němcová¹, E. Hřibová¹, M. Valárik¹, J. Čížková¹, L. Schillerová¹, A. Kilian² and J. Doležel¹

¹Laboratory of Molecular Cytogenetics and Cytometry, Institute of Experimental Botany, Olomouc, Czech Republic; ²Diversity Arrays Technology, Canberra, Australia

Despite the socio-economic importance of banana (*Musa* spp.), phylogenetic relationships within the family Musaceae, as well as the classification of subspecies and clones, remain subject to debate. Different types of molecular
markers have been used until now with various degrees of success, and a detailed picture is still lacking. An unexplored opportunity is to compare DNA sequences of a large set of genes across the Musaceae. In this work, we searched banana ESTs for homology to single-copy rice genes and hybridised candidates with banana genomic DNA. During the first phase of the project, sequences of 24 candidate genes from 14 carefully selected species (ITC collection, Belgium) representing maximal diversity within the Musaceae were analysed. The sequence analysis confirmed previously reported observations on clear divergence of *Musa*, *Ensete* and *Musella* as monophyletic groups. Moreover, within the *Musa* genus clade, section Eumusa clustered with Rhodochlamys, clearly separated from the cluster of Australimusa and Callimusa. These findings are in concordance with the previously reported close relation of these sections. The significance of these results was further supported by comparison with the results of nucleotide sequence analysis of the ITS1-5.8S-ITS2 region in selected Musaceae species as well as analysis with DArT markers. Although each of the methods used is based on a different principle, data that were collected and analysed supported the same concept of evolutionary relationship within the Musaceae species. We expect that a detailed analysis of the sequence data from higher number of genes and extension to other members of the family will clearly reveal phylogenetic relationships within the Musaceae and provide markers for unambiguous identification of subspecies and clones. This work was supported by the Grant Agency of the Academy of Sciences of the Czech Republic (grant award no. IAA600380703).

**Molecular Analysis Reveals Multiple Domestications of Edible Bananas**

H. Volkaert

*BIOTEC-National Science and Technology Development Agency, Thailand Science Park, KhlongLuang, PathumThanee 12120, Thailand, and Center for Agricultural Biotechnology, Kasetsart University, Kamphaengsaen, NakornPathom 73140, Thailand*

Molecular sequence analysis of six genes in a set of 100 cultivated and wild (*Musa acuminata* and *M. balbisiana*) banana accessions has been done to determine the origin of edible bananas and plantains. A few *Musa schizocarpa*
and Australimusa accessions were included as outgroups. The domestication of edible bananas involves three taxonomic groups: the *M. acuminata* subspecies *banksii* / *errans* group, the *M. acuminata* ssp. *malaccensis* / *microcarpa* / *zebrina* / *burmanica* / *siamea* group and the *M. balbisiana* group. Plantains (AAB) and several ABB bananas most likely originated through a hybridisation event between *M. acuminata* ssp. *errans* (or more generally the *M. acuminata* ssp. *banksii* group) and *M. balbisiana*. Most AAB bananas such as ‘Mysore’ and some BBA bananas such as ‘Pisang Awak’ are the result of hybridisation between *M. acuminata* ssp. “non-banksii” and *M. balbisiana*. Most AA and AAA edible bananas are derived from hybridisations between subspecies within the *M. acuminata* “non-banksii” group. Several unique SNPs have been identified in edible bananas and East African highland bananas (AAA) that so far have not been found in any of the wild *M. acuminata* accessions in the ITC collection. A search for these SNPs in the wild *Musa* populations would shed light on the original location of the hybridising populations that gave rise to the edible bananas. The variable contribution of parents at different genetic loci indicates that most edible bananas are not direct hybrids, but have gone through a few or several generations of back-crossing. Some diversity has been found in *M. balbisiana*. The *M. balbisiana* involved in the origin of plantains, other AAB bananas and the ABB ‘Monthan’ group is distinct from the *M. balbisiana* involved in the origin of the BBA ‘Pisang Awak’ group. A thorough study of the genetic diversity within *M. balbisiana* throughout its area of distribution is deemed necessary. The implications for breeding of edible bananas are discussed.

**Microsatellite Markers for Classifying and Analysing Genetic Relationship of Banana Cultivars in Indonesia**

A. Retnoningsih*, R. Megia and A. Hartana

*Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Indonesia; *Current address: Department of Biology, Faculty of Mathematics and Natural Sciences, Semarang State University, Indonesia*

Microsatellites were constructed as a molecular determination key for banana genomic groups. In this study, microsatellite markers were used to investigate genetic relationships between banana accessions collected from various areas in


Indonesia. One-hundred-sixteen (116) banana accessions were analysed using MaCIR108 and Ma-3-90 primers for identifying genomic groups and six additional microsatellite primers for genetic relationship analysis. Seventy-three accessions were classified into the AA or AAA genomic groups (\textit{Musa acuminata}), two accessions into the BB genomic group (\textit{M. balbisiana}), 21 accessions into the AAB genomic group and 20 accessions into the ABB genomic group. Ninety-nine accessions out of the 116 were unique genotypes and the rest were synonyms. The dendrogram generated by UPGMA separated the 116 banana accessions into two main clusters (coefficient of 0.13). All banana accessions belonging to the BB, AAB and ABB genomic groups clustered in the first main cluster, together with the majority of the accessions with a pure \textit{acuminata} genome. The second main cluster grouped 11 banana accessions with AA or AAA genome. All banana accessions containing the B genome were clustered according to their genomic groups, except four accessions of the AAB genomic group which clustered with accessions containing the A genome alone. The ABB genomic group appears closer to the BB than to the AAB genomic groups. The AA and AAA banana accessions could not be significantly distinguished, although the majority of their accessions tend to be clustered according to their ploidy level.

Validation of Rapid (Colour-Based) Pre-Screening Techniques for Analysis of Fruit Provitamin A Contents in \textit{Musa} spp.

L.C. Pereira\textsuperscript{1}, G.N. Newilah\textsuperscript{2}, M.W. Davey\textsuperscript{3} and I. Van den Bergh\textsuperscript{1}

\textsuperscript{1}Bioversity International, Parc Scientifique Agropolis II, 34397 Montpellier Cedex 2, France; \textsuperscript{2}Centre africain régional de recherches sur bananiers et plantains (CARBAP), 110 Rue Dinde Bonanjo, 832, Douala, Cameroon; \textsuperscript{3}Laboratory for Fruit Breeding and Biotechnology, Department of Biosystems, Katholieke Universiteit Leuven, De Croylaan 42, Heverlee, Leuven, Belgium

Banana and plantain (\textit{Musa} spp.) fruits have been shown to be a potentially rich source of provitamin A carotenoids (pVACs) and can thus play a key role in reducing vitamin A deficiency (VAD) in developing countries. Recently, the screening of over 170 \textit{Musa} genotypes indicates that there is substantial genetic diversity in the pVACs contents of banana and plantain fruit pulp. This knowledge is important as it can be used to encourage banana producers and
consumers to adopt high-pVACs cultivars as a sustainable means to combat VAD. The results can further help to focus *Musa* breeding programmes aimed at vitamin A biofortification. An additional screening of the more than 6,000 accessions maintained in over 60 field genebanks worldwide is highly desirable, but detailed pVACs analysis by spectrophotometry and HPLC is both time consuming and expensive, due to the need for specialised equipment and technical expertise. The aim of this work was to validate alternative pre-screening techniques for measuring fruit pVACs contents based on colour charts. These rapid methods use relatively inexpensive tools and do not require a high level of technical expertise. They could therefore be used to quickly pre-screen entire field germplasm collections and to select only the most interesting accessions for full pVACs analysis. It is expected that this will greatly reduce the costs and time required to screen the entire *Musa* genepool for pVACs content.

**Micronutrients Biofortification in *Musa*: Status, Bottlenecks and Prospects**

M.W. Davey¹, J. Keulemans¹, N. Roux² and I. Van den Bergh²

¹Laboratory of Fruit Breeding and Biotechnology, Department of Biosystems, Katholieke Universiteit Leuven, Belgium; ²Bioversity International, Parc Scientifique Agropolis II, 34397 Montpellier Cedex 2, France

Iron (Fe), Zinc (Zn) and vitamin A (vit A) are the major micronutrient deficiencies affecting large populations in developing countries worldwide. These deficiencies lead not only to an increased mortality and susceptibility to diseases, but also to a reduced capacity for work and development. Within sub-Saharan Africa and Asia, bananas, including plantains and cooking bananas (*Musa* spp.) are a primary staple food with per capita consumption levels reaching levels of up to 137 kg/year. Banana fruits are thus a major source of energy and essential (micro-)nutrients for these populations. While Fe and Zn-contents appear to be comparatively low in *Musa*, recent work has demonstrated that there is a huge range of variation in the fruit provitamin A carotenoids contents (pVACs), with levels approaching those found in the best-performing sweet potatoes and carrot varieties. It is thus clear that new (or non-indigenous) *Musa* varieties, rich in pVACs, have great potential to tackle the
problems of vitamin A deficiencies in these regions, in a cost-effective and sustainable manner. Here, we review the current status of our understanding of the genetic diversity of Musa pVACs contents and outline possible strategies and bottlenecks for the introduction and development of pVACs-rich Musa cultivars.

Candidate Gene Discovery: Resistance Gene Analog Characterisation and Differential Gene Expression Analysis in Musa-Mycosphaerella Host-Pathogen Interactions

R.N.G. Miller¹,², M.A.N. Passos¹, F.L. Emediato¹, C. de Camargo Teixeira¹ and G.J. Pappas Jr¹,³

¹Universidade Católica de Brasília, Brasília, DF, Brazil; ²Universidade de Brasília, Departamento de Biologia Celular, Brasília, DF, Brazil; ³EMBRAPA Recursos Genéticos e Biotecnologia, Brasilia, DF, Brazil

Many banana cultivars (Musa spp.) are sterile triploids or diploids, evolving only via somatic mutation. As a consequence, this crop generally lacks resistance to pests and diseases. Numerous disease resistance genes (R-genes) have been characterised in plants, conferring resistance to bacteria, viruses, fungi and nematodes. Identification and cloning of R-genes in Musa will provide new opportunities for genetic improvement. Our group has identified over 50 distinct NBS-LRR-type resistance gene analogs (RGAs) in the resistant wild diploid M. acuminata ssp. burmannicoides ‘Calcutta 4’. Characterisation is ongoing in M. acuminata-derived cultivars contrasting in resistance to Mycosphaerella leaf spot diseases, focusing on both the NBS-LRR R-gene family and cytoplasmic receptor-like kinases (RLKs) with extracellular LRRs. NBS-LRR class RGA probes applied to ‘Calcutta 4’, ‘Grande Naine’ (AAA, Cavendish subgroup), and M. balbisiana ‘Pisang Klutuk Wulung’ BAC libraries have revealed many putative resistance loci. Sequence data for such selected clones will provide insight into organisation and evolution of this R-gene class in Musa. Candidate gene discovery is also ongoing via analysis of differential gene expression from infected leaf cDNA during Musa-Mycosphaerella interactions, using both Sanger and Next Generation Sequencing approaches. Candidate R-genes will be applicable for banana genetic improvement via both plant transformation and conventional breeding using marker-assisted selection.
MA-ACS1: a Key Operator in Ethylene Biosynthesis in Banana - Its Role and Regulation during Fruit Ripening

S.R. Choudhury¹, S. Roy², S.K. Singh¹ and D.N. Sengupta¹

¹93/1, A.P.C. Road, Department of Botany, Bose Institute, Kolkata 700009, India; ²93/1, A.P.C. Road, Department of Chemistry, Bose Institute, Kolkata 700009, India

MA-ACS1 is the major ripening protein in banana and plays crucial role in the regulation of ethylene production during ripening. We identified the cDNA for MA-ACS1 from banana and studied the transcript and protein accumulation patterns of this gene. The expression of this gene correlated well with ACC content during ripening. In-silico analysis provided important insight in the sequential, structural and phylogenetic characteristics of MA-ACS1. We analysed the differential transcript accumulation pattern and protein levels of MA-ACS1 in response to ethylene, auxin, wounding and low temperature in preclimacteric banana fruit. Our study provides interesting information about the regulation of expression of the MA-ACS1 in response to various factors during ripening in banana fruit, which may have physiological relevance concerning ethylene biosynthesis during post-harvest conditions. Furthermore, we have detected a GCC-box putative ERE (ethylene-responsive element) and an ARE (auxin-responsive element) specific DNA-binding activity in the banana pulp and studied the ethylene and auxin responsive characteristics of the GCC-box and ARE (TGTCTC) containing synthetic promoter fragments. Finally, in the ripe banana pulp tissue, we detected a putative 40-kDa kinase activity that was found to specifically phosphorylate the MA-ACS1 as part of the post-translational modification of the protein. Understanding the expression pattern of ripening genes and the molecular mechanisms that regulate gene expression during ripening is crucial in regulating the expression of ripening genes to control ripening under post-harvest conditions to reduce massive spoilage of fruits during shipping and storage.
Combination of Suppression-Subtractive Hybridisation with cDNA Microarray, a Novel Way to Identify Genes from Banana Involved in Fruit Ripening, Quality Improvement and Tolerance to Stress

Z.Q. Jin\textsuperscript{1,2} and B.Y. Xu\textsuperscript{2}

\textsuperscript{1}Haikou Experimental Station, Chinese Academy of Tropical Agricultural Sciences, Hainan, P.R. China; \textsuperscript{2}Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Hainan, P.R. China

The isolation of mRNA transcripts-encoding proteins associated with physiological processes is a powerful tool for better understanding the mechanisms behind the processes. In a previous study, a forward suppression-subtractive hybridisation (SSH) cDNA library was constructed to isolate differentially expressed genes at early stage of post-harvest banana ripening. SSH was performed with cDNA from banana fruit on the day of harvest as the “driver” and cDNA from banana fruit 2 days post-harvest (DPH) as the “tester.” A total of 289 clones in the SSH library were sequenced. BLASTX results revealed that 191 cDNAs had significant sequence homologies with known sequences in the NCBI database. Of the 191 cDNAs, 138 were singletons and 53 belonged to divergent clusters containing 2-8 sequences. The identified cDNAs-encoding proteins are involved in cellular processes, such as metabolism, protein destination and storage, protein synthesis, signal transduction, transport and intracellular traffic, cell structure, growth and division, transcription and post-transcription, and disease and defence. To characterise differentially expressed cDNAs in the SSH library, cDNA microarray analysis was conducted. A total of 26 cDNAs in the 2-DPH banana fruit were found to be upregulated, and these results were confirmed by using reverse transcriptase-polymerase chain reaction (RT-PCR). Based on the information obtained in the study, the involvement of some of the genes in fruit ripening, quality improvement and tolerance to stress was confirmed.
Developing Resistant Banana and Plantain Cultivars through Conventional Breeding Techniques

R. Menon, A. Cherian, A. Suma, A. Maicykutty, P. Mathew, S. Nair and K.C. Aipe

Banana Research Station, Kerala Agricultural University, Kannara, Thrissur 680652 Kerala, India

Kerala, endowed with a warm humid tropical climate, is home to a wide spectrum of edible bananas, which include ‘Nendran’ (AAB), ‘Poovan’ (AAB), ‘Rasthali’ (AAB), ‘Chenkadali’ (AAA), ‘Neypoovan’ (AB), ‘Karpooravalli’ (ABB) and ‘Monthan’ (ABB). Their cultivation is constrained by various diseases and pests, such as Sigatoka leaf spot, Fusarium wilt, rhizome and stem weevils, nematodes and viral diseases. Conventional breeding directed at the development of resistant hybrids is in progress at the Banana Research Station, Kannara. Based on earlier breeding initiatives, two dessert banana hybrids, ‘BRS-1’ [‘Agniswar’ (AAB) x ‘Pisang Lilin’ (AA)] and ‘BRS-2’ [‘Vannan’ (AAB) x ‘Pisang Lilin’ (AA)], have been developed and released for cultivation in Kerala. These hybrids are extremely resistant to Sigatoka leaf spot, a trait inherited from the male parent. Current breeding strategies are focused on the improvement of ‘Nendran’, the commercial French plantain cultivar of the state, and envisage imparting higher productivity, dwarf stature and resistance to Sigatoka leaf spot, weevil borers and nematodes utilising wild/natural/bred diploids. Though limited by low levels of female fertility, development of plantain hybrids resistant to black leaf streak based on triploid/diploid crosses has been reported. Female fertile clones of ‘Nendran’ identified in the genebank were pollinated with the wild diploid *Musa acuminata* ssp. *burmannicoides* ‘Calcutta-4’, highly resistant to Sigatoka leaf spot. Low seed set and germination reduced the recovery of hybrids for evaluation. One hybrid progeny resulting from the cross ‘Nendran’ clone ‘Chengalikodan’ x ‘Calcutta 4’, with close resemblance to the female parent, recorded a plant height of 360 cm, pseudostem girth of 62 cm, 12 leaves at shooting and a bunch weight of 13 kg with 8 hands and 90 fruits. Unlike its susceptible female parent, the hybrid displayed very high resistance to Sigatoka, imparted by the male parent. Being male and female fertile, the hybrid is being back-crossed with ‘Nendran’ and crossed with selected diploids. Embryo culture is being standardised to improve the recovery of hybrid progeny.
Exploitation of Diploids in Indian Banana-Breeding Programmes

S. Uma, M.M. Mustaffa, M.S. Saraswathi and P. Durai

National Research Centre for Banana (NRCB-ICAR), Thogamalai Road, Thayanur (Post), Tiruchirapalli 620102, Tamil Nadu, India

Banana is a recalcitrant crop for improvement due to inherent plant-based constraints like polyploidy, parthenocarpy and male and/or female sterility. Success of banana breeding depends mainly on the use of natural diploids and/or developing superior synthetic diploids. India has nurtured the development of a number of wild and parthenocarpic diploids harbouring resistant gene sources for a number of biotic and abiotic factors. Present study on 18 diploids includes eight parthenocarpic and six wild diploid accessions of which ten are indigenous and four are exotic introductions. They have been evaluated for various traits like resistance to nematodes, weevils and borers, Fusarium wilt, leaf spot complex and reaction to drought. Based on their usefulness of traits, diploids were included in the breeding programme either directly or through development of synthetic diploids. This paper reports on the results of evaluation of these 18 diploids for their fertility status, breeding behaviour and compatibility status with other diploid and triploid parents. The paper also provides the baseline information on factors affecting seed set, like temperature and relative humidity, extent of seed set in 79 diploid cross combinations, percent of good seeds with viable embryos, germination time (10-432 days), germination success (0-92%) and regeneration capacity (2.5-98.3%). This baseline information will be useful in selecting the parents for future breeding programmes with better success in seed yield. The poor germination and regeneration of hybrid seeds into plantlets reiterates the need for standardised techniques for embryo rescue for better success in improvement of banana through classical breeding.
Breeding Increases Genetic Diversity of East African Highland Bananas (*Musa* spp.): an Assessment Using Molecular Tools

N. Moses$^1$ and M. Pillay$^{1,2}$

$^1$International Institute of Tropical Agriculture, PO Box 7878 Kampala, Uganda;  
$^2$Current address: University of South Africa, Private Bag X6, Florida 1710, Gauteng, South Africa

The development of high-yielding, disease-resistant varieties through plant breeding has led to the replacement of farmer-developed varieties with uniform bred varieties in many crops. The adoption of these uniform varieties is one of the main factors that have contributed to the reduced loss of on-farm genetic diversity. The East African highland banana (EAHB, AAA genome) is a staple food crop and source of income for millions of people in countries surrounding the Great Lakes region of East Africa. Studies have indicated a low level of genetic diversity among the EAHB varieties. Reduced yields due to the major diseases (black leaf streak, Fusarium wilt, Xanthomonas wilt) and pests (nematodes and the banana weevil) is threatening the livelihoods of millions of people who depend on EAHB for food and income. Due to the absence of resistance genes among the triploid EAHB varieties, genetic improvement of the crop can only be achieved by breeding. Banana breeding programmes have developed a number of banana hybrids over the years. However, no attempt was made to compare the genetic diversity of the progeny to that of their parents. The objectives of this study were to: (i) assess the genetic diversity of fourteen banana hybrids derived from crosses between eight EAHBs and four diploids, and (ii) demonstrate that banana breeding can increase rather than reduce the diversity of the crop. In this study, we demonstrate that the inherent nature of banana breeding increases genetic diversity using RAPD (random amplified polymorphic DNA) and AFLP (amplified fragment length polymorphism) techniques.
Use of Molecular Markers in Banana and Plantain Improvement

J. Lorenzen\textsuperscript{1}, S. Hearne\textsuperscript{1}, G. Mbanjo\textsuperscript{1}, M. Nyine\textsuperscript{1} and T. Close\textsuperscript{2}

\textsuperscript{1}International Institute of Tropical Agriculture; \textsuperscript{2}University of California, Riverside, USA

Banana and plantain (\textit{Musa} spp.) are a key crop for food security and livelihoods in Africa, particularly in the Great Lakes region of highland East Africa where they are a major staple food. Originating from Asia, banana and plantain spread across Africa many centuries ago. Increased global movement of plants in the past 150 years introduced pests and diseases from other regions that became new constraints to banana production in Africa. These biotic challenges to banana production include fungi, bacteria, viruses, nematodes and insects. Most banana and plantain in Africa are grown under low levels of crop management in which water is a primary limitation in most rain-fed cropping systems. There is wide scope for improving banana through introgression of host-plant resistance to both biotic and abiotic challenges. Given the slow and land-intensive nature of \textit{Musa} breeding, there is tremendous potential to accelerate the process through marker-assisted breeding (MAB). Since banana has been relatively neglected in terms of development of molecular tools, we have been adding some basic tools for mapping and germplasm characterisation. In the past 2 years, we have characterised over 400 new EST-based SSR markers, half of which should be mapped this year. An EST sequencing project was complemented by bioinformatics processing for SNP detection and a new tool for visualisation. Characterisation of SNPs in candidate genes is in progress. The imminent advent of a reference genome sequence for \textit{Musa} should accelerate the progress of MAB, expanding the number of available loci for marker development and increasing focus on mapping traits and characterising diversity. Future maps will benefit from higher marker density, high speed assays and lower costs per data point, especially if we are able to realise greater cost efficiencies through international collaborative efforts.
The Use of Molecular Markers in CIRAD’s Current Banana Breeding Programme

J-P. Horry

Centre de coopération internationale pour la recherche et le développement (CIRAD), Montpellier, France

For several years, CIRAD (Centre de coopération internationale pour la recherche et le développement) has invested in the genetic improvement of banana and plantain with the goal to sustain production with better respect for the environment for both export trade and local consumption. An original crossing strategy was developed, aimed at the development of triploid hybrid varieties directly from diploid plant material. Molecular tools have been developed to support this conventional cross-breeding scheme. Molecular markers have given a new insight in *Musa* genetic diversity, leading to a better understanding of the lineage between ancestry and modern cultivars, thereby providing critical information for the genitor’s choice. The *Musa* genome structure and genetic maps have been investigated, providing a better knowledge of the mating behaviour of gametes during meiosis and of how to manage ploidy and interspecificity. Genes involved in character expression have been identified, and an international effort to sequence the genome is ongoing. The development of these tools provides breeders with important information increasing the efficiency of genetic improvement efforts. This paper presents how the molecular approach is actually implemented in CIRAD’s breeding strategy.
Development of Highly Regenerative Embryogenic Cell Suspensions of Banana Cultivar ‘Nanjangud Rasbale’ (syn. ‘Rasthali’, AAB, Silk Subgroup) and Transformants with AMP Gene

S. Mohandas, H.D. Sowmya, R. Manjula, K.Y. Pratibha, M. Manamohan and S. Meenakshi

Division of Biotechnology, Indian Institute of Horticultural Research, Hessaraghatta, Bangalore 560089, India

Banana cultivar ‘Nanjangud Rasbale’ (syn. ‘Rasthali’, AAB, Silk subgroup), one of the special banana cultivars of Karnataka State in India, is threatened by Fusarium wilt caused by Fusarium oxysporum f. sp. cubense (Foc). Genetic transformation techniques have shown great potential for inducing resistance to Foc. Regeneration through somatic embryos developed from embryogenic cell suspension (ECS) culture is one of the popular methods to transform banana. There is no report of the development of highly regenerative suspension cultures from this cultivar using floral meristems. Floral meristems were cultured on callus induction medium containing 2,4-D 4 mg/L. Embryogenic calli were selected and cell suspensions were developed and maintained on 2,4-D 1 mg/L. The cell suspensions plated on media containing BAP 1 mg/L and IAA 2mg/L developed into embryos in 45 days and germinated in 60 days. One ml culture of the suspension produced 2080-2960 somatic embryos which produced 1500-2000 plantlets. Histological observations confirmed that 75% of the embryos were normal with shoot and root meristem, while 25% showed various levels of abnormalities. After standardising different transformation conditions, the ECS (0.5 OD) was cocultivated with Agrobacterium containing AMP gene in pCAMBIA 2301 vector for 30 minutes with 100 µM acetosyringone and plated on 50 mg/ml G418. Somatic embryos developed and regenerated on same selection medium with a total germination of 12%. RT-PCR, Dot blot and Southern blot assay of the regenerants showed integration of the gene in 10 out of 17 plants tested. Further evaluation of the transgenics is in progress.
Opening of Workshop 1: Opportunities for Bridging the Gap between Genomics and Genetic Improvement in *Musa* spp.

N. Roux¹, M. Smith², M. Rouard¹, X.L. Huang³ and the GMGC consortium

¹Commodities for Livelihoods programme, Bioversity International, Parc Scientifique Agropolis II, 34397 Montpellier Cedex 5, France; ²Department of Primary Industries & Fisheries, Maroochy Research Station, 47 Mayers Road, Nambour, Qld 4560, Queensland, Australia; ³School of Life Sciences, Zhongshan (Sun Yat-Sen) University, Guangzhou, China

Bananas and plantains (*Musa* spp.) represent the fourth most important commodity crop in the world, important for export but also for food security in developing countries. The crop is susceptible to an ever-increasing range of pests and diseases, with commercial cultivars heavily dependent upon pesticides which are associated with adverse environmental and health impacts, and which threaten the sustainability of the crop. There is an urgent need to develop improved banana cultivars with a wider range of pest and disease resistance, but conventional breeding is hindered by low fertility of the crop, structural heterozygosity and polyploidy. The Global *Musa* Genomics Consortium (GMGC, http://www.musagenomics.org) is an international network of investigators committed to improve understanding of genomic evolution in relation to biotic and abiotic stresses in a polyploid, vegetatively propagated crop, with the aim to underpin traditional breeding efforts with genomic tools. The role of GMGC is to provide meaningful insights for the plant community. Indeed, *Musa* lies taxonomically within the monocots, although distant from the grass family (Graminiaceae), in a position that is important for comparative and evolutionary genomics. The Consortium currently brings together expertise from 38 institutions in 24 countries. Members are committed to close collaboration and agree to share materials and resources, including sequence data and enabling technologies. Wherever possible, the products of the Consortium are placed in the public domain. With the *Musa acuminata* genome to be fully sequenced next year, it is essential that the community be ready to use this information as efficiently as possible. Therefore this presentation will explore strategies such as the development of markers, gene discovery (e.g. resistant gene analogs), comparative genomics, gene function confirmation and bioinformatics based on whole-genome sequence information to support breeding programmes.
Keynote 1: Studies on Some of the Early Events in the *Fusarium oxysporum*-*Musa* Interaction


*Fruit Tree Research Institute, Guangdong Academy of Agricultural Sciences, Guangdong Province, China*

*Fusarium oxysporum* f. sp. *cubense* (Foc) is the causal pathogen of Fusarium wilt in banana. A better understanding of the many factors that play an important role in the interaction between the pathogen and its host may contribute to developing novel strategies to control the disease. Firstly, root exudates are associated with the resistance or sensitivity of Cavendish banana to Foc race 4. A chemotactic response of the spores of the soil-inhabiting plant-pathogenic fungus to root exudates of susceptible banana plantlets was observed, indicating that a highly potent attractant exists in the root exudates. Interrupting the chemotactic response may be an effective way to control the disease. On the other hand, the germination of spores and hyphal growth of Foc race 4 was inhibited significantly by the root exudates of wilt-resistant banana cultivars, compared with untreated spores and spores treated with root exudates from susceptible cultivars, which suggests that this inhibitor in root exudates of resistant plants might be developed as a botanical fungicide. Secondly, the pathogen is predicted to produce an elicitor. Activity of chitosan oligosaccharides, peroxidase, β-1,3-glucanase and phenyl-alanine-ammonia-lyase activity was increased more remarkably and rapidly in the roots of resistant cultivars treated with the elicitor than in the roots of treated sensitive cultivars, suggesting that an elicitor might be used to induce resistance. Thirdly,
mycotoxins are predicted as virulence factors for Foc: fusaric acid can induce necrosis, while the potential role of beauvericin in pathogenesis is still unknown. Fourthly, a green fluorescent protein-tagged Foc strain was developed and its pathogenesis studied with fluorescence microscopy and confocal laser scanning microscopy. Based on the results, a rapid, efficient evaluation method was developed, with which resistant cultivars of the Chinese Banana Germplasm Collection could be identified.

**Keynote 2: Fusarium Wilt of Banana – Renewed Threat and Renewed R&D Interest**

A.B. Molina

*Commodities for Livelihoods programme, Bioversity International - Asia Pacific, 3F Khush Hall, IRRI, Los Banos, Laguna, Philippines*

Following the Fusarium wilt-induced demise of popular banana cultivar ‘Gros Michel’ (AAA) and its consequent expensive but successful substitution by the race 1-resistant cultivars of the Cavendish subgroup in the 1950s, research and industry interests on its pathogen, *Fusarium oxysporum* f. sp. *cubense* (Foc), have dwindled, although race 1 remains a major production constraint of many locally important cultivars. However, the recent epidemic of Fusarium wilt caused by tropical race 4 (TR4) on Cavendish plantations in Asia has raised new concerns within the banana industry, more specifically about the sustainability of the Cavendish-based export banana industry. While TR4 has been ravaging banana production in Taiwan, Indonesia and Malaysia for some time, within the last two decades new epidemics in China and the Philippines have renewed the concerns of the industry and generated new research interests. More than 90% of the total banana production in China is Cavendish, whilst the Philippines is the top Cavendish banana exporter in Asia and world’s number two exporter after Ecuador. Similarly, TR4 is a pathogen of transboundary relevance to the huge Cavendish banana industry of Latin America, and also Africa. In addition, the impact of TR4 is even more serious, as it also attacks many other cultivars grown by small-scale farmers. This paper will provide an overview of Fusarium wilt as an export industry problem and a threat to the livelihoods of small-scale banana farmers. It will review current disease threat mitigation activities by the industry and within national
programmes, including some disease management strategies and tactics being used or tried. Some relevant research areas being pursued in Asia are also discussed. The paper also examines activities on preventive and preparatory measures, such as raising awareness of various stakeholders in countries where TR4 is not yet found, including Latin America, the major producer of Cavendish for the global trade. Finally, the paper also assesses the prospects of the banana industry and small-scale producers in the light of the TR4 threat, and of current knowledge and technologies, including tissue-culture technology, breeding methods, policies and new production systems.

Incidence and Distribution of Fusarium Wilt in Indonesia

C. Hermanto¹, A. Sutanto¹, Jumjunidang¹, H.S. Edison¹, J. Daniells², W. O’Neill³, V.G. Sinohin⁴ and A.B. Molina⁴

¹Indonesian Tropical Fruit Research Institute, Jl. Raya Solok - Aripan km 08, PO Box 5, Solok 27301, West Sumatera, Indonesia; ²Department of Primary Industries and Fisheries, PO Box 20, South Johnstone 4859, Queensland, Australia; ³Department of Primary Industries and Fisheries, 80 Meiers Rd, Indooroopilly 4068, Queensland, Australia; ⁴Commodities for Livelihoods programme, Bioversity International - Asia Pacific, 3F Khush Hall, IRRI, Los Banos, Laguna, Philippines

Fusarium wilt is a major banana production constraint in Indonesia. Field observations and sample collections, facilitated by two Australian Centre for International Agricultural Research (ACIAR)-funded projects, were undertaken to generate a VCG-based Fusarium wilt distribution map. The activities were conducted from 2006 to 2009 in 15 banana-producing provinces, where each province involved two districts, each encompassing two banana-producing areas. Fifteen to 20 banana orchards were observed, and vascular strand samples were collected from the same fields. Incidence of Fusarium wilt ranged from 0.14 to 100%, with an average of 25.16% ± 16.02. Seven VCGs of Fusarium oxysporum f. sp. cubense (Foc) were initially identified, namely VCG 0120, 0120/15, 01213/16, 01218, 0123, 0124/5 and 0126. One third of the isolates was identified to be Foc tropical race 4 (TR4) VCG 01213/16. TR4 was distributed widely in almost all provinces, infecting local cultivars ‘Barangan’ (AAA), ‘Raja’ (AAB) and ‘Ambon Hijau’ (AAA), as well as other cultivars of different ploidy and genome groups. Beside improper management by banana
farmers, the wide distribution of the disease may be due to movement of planting materials along with transmigration of people from Java to other islands.

Status of Fusarium Wilt Research in India

M.M. Mustaffa and R. Thangavelu

National Research Centre for Banana (NRCB-ICAR), Thogamalai Road, Thayanur (Post), Tiruchirapalli 620102, Tamil Nadu, India

With an annual production of 22 million tonnes, India is the world’s largest producer of banana. Many pests and diseases cause huge economic losses to the farmers. Among these, Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* (Foc) is one of the most important production constraints and is widespread in susceptible cultivars with 80-90% severity in many banana growing states. The important groups of banana severely affected are Silk’ (AAB), ‘Neypoovan’ (AB), ‘Pisang Awak’ (ABB), ‘Pome’ (AAB), ‘Bluggoe’ (ABB) and ‘Monthan’ (ABB). It was recently found that ‘Mysore’ (AAB) is also susceptible to Foc (VCG 0124/5). Recently, a virulent strain of Foc affecting Cavendish types has been identified, the so-called race 4. In order to find out the various pathotypes in Foc, a VCG analysis was carried out in 200 Foc isolates collected from different parts of India. The analysis indicated the presence of six different VCG groups (0124, 0125, 0124/5, 0128, 01218, 01220) belonging to race 1 and 2, but absence of VCG groups of race 4. No effective control measures have been developed worldwide, except growing of resistant cultivars. Recently, the NRC Banana has identified an effective fungal antagonist *Trichoderma viride* which can effectively control the soil-borne inoculum of the Fusarium pathogen. A mass production protocol at farm level using farm waste materials has been standardised for cost-effective management of the disease. Recently, molecular markers for the identification of pathogenic *Fusarium* present in the soil as well as in the planting materials have been developed. Presently, research activities on diversity analyses of Foc, pathogen-host resistance, biological control using endophytes, standardisation of diagnostic kit for the identification of pathogen present in the soil and in the plant are the major thrust areas of research in India. Activities for the effective management of this disease are discussed.
Raising Awareness of the Threat of Tropical Race 4 of Fusarium Wilt for Latin America and the Caribbean

L.E. Pocasangre¹, R. Ploetz², A.B. Molina³ and L. Perez Vicente¹

¹Commodities for Livelihoods programme, Bioversity International - Latin America and the Caribbean, c/o CATIE, Turrialba 7170, Costa Rica; ²University of Florida, 18905 SW 280th Street, Homestead, Florida 33031-3314, USA; ³Commodities for Livelihoods programme, Bioversity International - Asia Pacific, 3F Khush Hall, IRRI, Los Banos, Laguna, Philippines

Banana and plantain are among the most important commodities, both as staple food and as export crop, for many countries in Latin America and the Caribbean. Tropical race 4 (TR4) of *Fusarium oxysporum* f. sp. *cubense* (Foc), which is attacking bananas in Asia, represents a threat to the export banana industry which in Latin America is based almost 100% on the Cavendish subgroup, which is susceptible to Foc TR4. Production for national markets, based on plantain, apple banana and ‘Gros Michel’, is also under threat. Bioversity International has formed a strategic alliance with the research and development network for plantain and banana for Latin America and the Caribbean (MUSALAC), the Organismo Internacional Regional de Sanidad Agropecuaria (OIRSA), the University of Florida and the Instituto Nacional de Investigacion de Sanidad Vegetal (INISAV) to launch an awareness campaign in order to prevent the entrance of Foc TR4 into the Americas. The threat of Foc TR4 was first raised in the VII steering committee meeting of MUSALAC held in Panama in October 2007. The country representatives prepared a resolution emphasising the importance of quarantine measures, which has been distributed to all 13 country members of MUSALAC. In addition, OIRSA sent an official communication to the agriculture ministries of nine countries in Central America in order to strengthen the quarantine measures of the plant protection departments to prevent the entrance of this pathogen. Bioversity International and OIRSA are also co-organising a training workshop and expert consultation with country quarantine officers and international Fusarium specialists. The workshop provides technical training to country representatives and input into the preparation of quarantine guidelines oriented to all stakeholders of the banana production chain and related sectors.
Development of a Detection Method for Tropical Race 4 of *Fusarium oxysporum* f. sp. *cubense*

C. Waalwijk¹, M.A. Dita¹,², I. Buddenhagen³, K., L.V. Paiva⁴, M.T. Souza Jr.¹,⁵ and G.H.J. Kema¹

¹Plant Research International, PO Box 16, 6700 AA Wageningen, The Netherlands; ²Embrapa Cassava & Tropical Fruits, Cruz das Almas, 44380-000, Bahia, Brazil; ³1012 Plum Lane, Davis, California, USA; ⁴Universidade Federal de Lavras, Caixa Postal 3037, Lavras-MG, Brazil; ⁵Embrapa LABEX Europe, PO Box 16, 6700 AA Wageningen, The Netherlands

*Fusarium oxysporum* f. sp. *cubense* (Foc) is the causal agent of Fusarium wilt, the devastating disease that ruined the ‘Gros Michel’ (AAA)-based banana production in the early 1900s. The occurrence of a new variant, called tropical race 4 (TR4), in Southeast Asia that overcomes the resistance in Cavendish clones such as ‘Grand Naine’ (AAA) is a major concern to current banana production worldwide. The threat posed by this new variant could be overcome by the introduction of resistant cultivars. However, the identification of new resistant sources or breeding for resistance is a long-term effort. At the moment, the only option to control the disease is to eradicate infected plants and isolate infested plantations, to avoid or reduce the spread of the pathogen. This requires a sensitive and highly specific diagnostic that enables early detection of the pathogen and prevents false positives as this will have dramatic economic consequences for the banana growers. A two-locus database of DNA sequences, from over 800 different isolates from multiple formae speciales of *F. oxysporum*, was used to develop a molecular diagnostic tool that specifically detects isolates from the Vegetative Compatibility Group (VCG) 01213, which encompasses the Foc TR4 genotypes. This diagnostic tool was able to detect all Foc TR4 isolates tested, while none of the Foc isolates from 19 VCGs other than 01213 showed any reaction. In addition, the developed diagnostic tool was able to detect Foc TR4 when using DNA samples from different tissues of ‘Grand Naine’ plants inoculated with TR4 isolates. Details on specificity, sensitivity and accuracy will be presented.
Characterisation of Isolates of *Fusarium oxysporum* f. sp. *cubense* into Vegetative Compatibility Groups in Brazil

A.P. de Matos¹, J.d.S. da Silveira², D.M.V. Ferreira³, Z.J.M. Cordeiro¹ and R.O. Trocoli

¹Embrapa Cassava and Tropical Fruits, Caixa Postal 007, Cruz das Almas, 44380-000, Bahia, Brazil; ²Escola de Agronomia da UFBA, Cruz das Almas, 44380-000, Bahia, Brazil; ³Agência de Defesa Sanitária da Bahia, Cruz das Almas, 44380-000, Bahia, Brazil

Fusarium wilt, caused by the fungus *Fusarium oxysporum* f. sp. *cubense* (Foc), is one of the most significant constraints of banana production worldwide. The disease occurs in all banana-growing regions of the world. Considered a highly complex pathogen, studies on Foc characterisation have been performed by distinct methods, including vegetative compatibility group (VCGs). In this study, 44 isolates of Foc from some banana-growing areas of Brazil were characterised using the VCG technique. Nit mutants of Foc were paired cultured with the following VCG standards: 0120, 0121, 0122, 0123, 0124, 0125, 0128, 0129, 01210, 01211, 01212, 01213, 01214 and 01215. Results showed that the Brazilian isolates of Foc evaluated in this work belonged to VCG 0124, VCG 0125, VCG 0120, VCG 01215, VCG 0128, VCG 0123, VCG 01210 and VCG 01215. Isolates Foc BA 8, Foc BA 12, Foc BA 28 and Foc BA 29 did not generate any heterokaryons when paired with the Foc standards. This is the first report of VCGs 0123, 0129 and 01215 in Brazil.
Vegetative Compatibility Group Analysis of Indonesian *Fusarium oxysporum* f. sp. *cubense* Isolates

W.T. O’Neill¹, L.M. Gulino¹, A.B. Pattison², J.W. Daniells², C. Hermanto³ and A. Molina⁴

¹Department of Primary Industries and Fisheries, 80 Meiers Rd, Indooroopilly 4068, Queensland, Australia; ²Department of Primary Industries and Fisheries, PO Box 20, South Johnstone 4859, Queensland, Australia; ³Indonesian Tropical Fruit Research Institute, PO Box 5, Solok 27301, West Sumatra, Indonesia; ⁴Bioversity International, c/o IRRI, Los Banos, Laguna 4031, Philippines

As part of an Australian Centre for International Agricultural Research (ACIAR)-funded project, vegetative compatibility group (VCG) analysis was used to characterise isolates of *Fusarium oxysporum* f. sp. *cubense* (Foc) collected from 12 Indonesian provinces. Samples were taken almost exclusively from cultivated bananas (around 20 different cultivars) in smallholder and backyard plots. In total, 178 isolates were received and, at the time of writing, 135 have been assigned a VCG. Isolates have so far tested positive for the following VCG groups: 0120/15, 0121, 0123, 0124/5, 0126, 01213/16, 01218 and 01219. All of these strains have previously been detected in Indonesia, and most results obtained in this project are consistent with those of previous workers who have studied the diversity and distribution of Foc in Indonesia. However, our knowledge of Foc diversity in poorly studied provinces, such as those in Kalimantan, has been increased by the results from this work. VCG 01213/16 (the so-called tropical race 4, TR4) has been by far the most prevalent strain detected, accounting for approximately 60% of the positive results. It was most commonly isolated from the cultivars ‘Barangan’ (syn. ‘Lakatan’, AAA) and ‘Raja’ (AAB). In this project, TR4 has been detected in Sumatra (Aceh and West Sumatra), Java (West Java and Daerah Istimewa Yogyakarta), Kalimantan (West and East Kalimantan), Sulawesi (South East and North Sulawesi) and Papua. These findings highlight the wide distribution of Foc TR4 in Indonesia and the importance of managing its spread. Large export Cavendish plantations established in Indonesia in the 1990s were abandoned due to the disease, and locally important commercial cultivars, such as ‘Barangan’, are especially vulnerable. Infected planting material continues to be moved to new areas (within and between provinces),
and TR4 is now found very close to the border of Papua New Guinea, posing an imminent threat to the huge banana diversity of that country.

Cross-Infection Potential of Fusarium Wilt Isolates and their Diversity Analysis by Vegetative Compatibility Grouping, Sequencing of rDNA-ITS region and rDNA-IGS- RFLP Analysis

R. Thangavelu, P. Suganya Devi and P. Maria Chrismala (presented by M.M. Mustaffa)

National Research Centre for Banana (NRCB-ICAR), Thogamalai Road, Thayanur (Post), Tiruchirapalli 620102, Tamil Nadu, India

Fusarium wilt, caused by Fusarium oxysporum f. sp. cubense (Foc), is posing a serious threat to banana production in India. Out of 234 Foc isolates collected from different parts of India, 100 representative isolates were subjected to cross reaction as well as diversity analyses, with the aim to develop effective management practices. In-vitro cross reaction tests involving different VCGs belonging to Foc race 1 and race 2 indicated that there was cross reaction between race 1 and race 2 VCGs in many cases. Validation of the cross-infection potential between Foc race 1 and race 2 isolates was carried out under pot culture conditions using ‘Rasthali’ (AAB) and ‘Bluggoe’ (ABB). The study confirmed the cross reaction between the members of Foc race 1 and race 2 as the Foc race 2 isolate 5MT obtained from ‘Monthan’ (ABB) caused wilt disease in ‘Karpuravalli’ (ABB) which is normally susceptible only to Foc race 1 isolates. For diversity analyses, a total of 141 nit mutants were developed from the 100 representative Foc isolates. The phenotypic characterisation of these 141 nit mutants indicated the presence of 9 nit-M testers, 75 nit-1 mutants and 57 nit-3 mutants. The VCG analyses carried out using these local nit-M testers as well as 33 nit-M testers obtained from Queensland Department of Primary Industries and Fisheries (QDPI), Australia indicated that there are nine different groups of Foc in India, of which six are of known VCG groups. The molecular characterisation of Foc isolates by sequencing rDNA-ITS region analysis and the phylogram drawn for these sequences also revealed the presence of nine major groups. The possible origin of all these isolates could be
the ‘Hill Banana’ (AAB). In the case of PCR-RFLP analysis of rDNA-IGS region, the six restriction enzymes used, namely HaeIII, Rsal, HhaI, Hinfl, TaqI and MspI, grouped the 100 Foc isolates into 13 IGS genotypes. Group 1 (AAAAAA) was the most common and consisted of 44 isolates of pathogenic *Fusarium*.

**Gene Expression Analysis in Roots of *Musa acuminata* ssp. *burmannicoides* ‘Calcutta 4’, a Resistant Genotype for Fusarium Wilt in Banana**

K.V. Ravishankar, A. Rekha, V. Swarupa and G. Savitha

*Indian Institute of Horticulture Research, Hesarghatta Lake Post, Bangalore 560089, India*

Fusarium wilt, caused by the fungal pathogen *Fusarium oxysporum* f. sp. *cubense* (Foc), is one of the most devastating diseases of banana. In India, most banana cultivars are highly susceptible to this pathogen. In this study, *Musa acuminata* ssp. *burmannicoides* ‘Calcutta 4’ (resistant) and cultivar ‘Kadali’ (AA; susceptible) were infected with Foc in pot experiments. ‘Calcutta 4’ showed significantly lower disease severity than ‘Kadali’, which showed symptoms like early yellowing of leaves, browning of roots and vascular discoloration of the corm. Suppression subtractive hybridisation was carried out to identify genes induced in roots of ‘Calcutta 4’ after infection with Foc. One hundred and nine (109) clones were sequenced and 68 non-redundant sequences were obtained. They were characterised based on homology search in the NCBI (National Center for Biotechnology Information) database. A few cDNAs which showed homology were PGIP-2, peroxidase, polyphenol oxidase and catalase, which play a role in defence mechanisms. A few genes like S-adenosyl methionine synthetase-1 and -2 and methyl transferases, which might be involved in the pathways of cell wall biosynthesis and signal transduction in response to Fusarium wilt, were also expressed.
**Application of gfp-Transformed *Fusarium oxysporum* f. sp. *cubense* for the Tracking of the Infection Process and the Evaluation of Resistant Banana Cultivars**


*Fruit Tree Research Institute, Guangdong Academy of Agricultural Sciences, Guangdong Province, China*

*Fusarium oxysporum* f. sp. *cubense* (Foc) is the causal pathogen of Fusarium wilt in banana. In order to understand its infection pattern and process for effective disease control, we developed a green fluorescent protein (gfp)-tagged Foc strain and studied its pathogenesis with fluorescence microscopy and confocal laser scanning microscopy. The fungal protoplasts were transformed by a polyethylene glycol/CaCl$_2$-mediated transformation method described by Tudzynski. The transformation was very efficient and gfp expression was stable for at least six subcultures, and the fluorescent signal was clearly visible in the hyphae and spores. The transformed Foc retained its pathogenicity and growth patterns. The experiment showed that: (i) Both microconidia and hyphae are capable of invading the host; (ii) The invasion sites include root hairs, root tips and natural wounds along the lateral root base, but not the highly lignified primary lateral root; (iii) In the root tissue, microconidia develop into germ tubes or directly degrade the cell wall; hyphae are able to penetrate directly cuticles and grow inside or outside cells; (iv) Invasive structures, including cone and invasion paths were observed; (v) Development of spores and hyphae in the root system, bulb and pseudostem was observed. Based on the results, we have developed a rapid, efficient evaluation method, with which we have evaluated the resistance of cultivars from the Chinese Banana Germplasm Collection. The results of this work demonstrate that gfp is an effective tool not only for the study of plant-fungus interactions, but also to support the breeding of resistant banana cultivars.
Characterisations of Early Events in the Fluorescent-Tagged
*Fusarium oxysporum* f. sp. *cubense* and Banana Root Interaction

X.M. Yin¹, W. Zheng², B.Y. Xu³, J.B. Wang⁴ and Z.Q. Jin¹,³

¹HaiKou Experimental Station, Chinese Academy of Tropical Agricultural Sciences (CATAS), Haikou, China; ²College of Agriculture, Hainan University, Danzhou, China; ³Tropical Crops Biotechnology Institute, CATAS, Haikou, China; ⁴Environment and Plant Protection Institute, CATAS, Danzhou, China

The infection of banana by *Fusarium oxysporum* f. sp. *cubense* can result in highly variable disease symptoms ranging from asymptomatic plants to severe rotting and wilting. We developed a green fluorescent protein-tagged strain and used it to study the banana root colonisation and infection process in vivo. Using confocal laser scanning microscopy, colonisation, infection and disease development in banana roots were visualised in detail. The complete colonisation pattern of the banana root system was progressive, the pathogenesis occurred from the root surface to the root mesocotyl, and from the roots to the centre of the pseudostem. The very first contact between the fungus and the host took place at the root hair zone by mingling and by the attachment of hyphae to the banana root. The preferential colonisation sites to the root surface were the grooves along the junction of the epidermal cells. A mosaic pattern of infection resulted from specific epidermal and cortical cells becoming infected by intercellular hyphae while surrounding cells were uninfected. Specific infection sites, such as sites of root hair, root tips or wounded tissue, and specific infection structures, such as appressoria, were absent. These observations confirm existing theories of banana-Foc interaction and further enrich our knowledge of the infection mechanism of *F. oxysporum*. The results prove that gfp can be used as convenient, fast and effective marker for studying banana-fungus interactions.
A Greenhouse Bio-Assay for the *Fusarium oxysporum* f. sp. *cubense* (Tropical Race 4) x Banana (Cavendish Subgroup) Interaction

M.A. Dita$^{1,2}$, C. Waalwijk$^2$, L.V. Paiva$^3$, M.T. Souza Jr$^{2,4}$ and G.H.J. Kema$^2$

$^1$Embrapa Cassava & Tropical Fruits, Cruz das Almas, 44380-000, Bahia, Brazil; $^2$Plant Research International, 6708 PB, Wageningen, The Netherlands; $^3$Universidade Federal de Lavras, Caixa Postal 3037, Lavras- MG, Brazil; $^4$Embrapa LABEX Europe, 6708 PB, Wageningen, The Netherlands

Several disease resistance screening protocols for Fusarium wilt of banana (causal agent *Fusarium oxysporum* f. sp. *cubense* - Foc) under greenhouse conditions have been reported. However, a standard and worldwide accepted methodology is still undefined. A standardised greenhouse bioassay for this pathosystem is necessary for the identification of resistant banana genotypes and for detailed plant-pathogen interaction studies. This became especially important with the advent of tropical race 4 (TR4), which is a significant threat for the global Cavendish-based banana export industry. This work aimed to establish a reliable and rapid greenhouse bioassay for Foc with a focus on the TR4-Cavendish interaction. Using a double-pot system, hardened 3-months-old tissue-culture plants of ‘Grand Naine’ (AAA, Cavendish subgroup) were, separately, inoculated with three isolates, belonging to the Vegetative Compatibility Group (VCG) 01213 and considered as TR4, and one isolate, ranked as race 1 with known pathogenicity on ‘Silk’ (AAB). Plants were inoculated by root dipping (30 minutes, 105 conidia/mL) and then transferred to pots partially filled with sand supplemented with 20 maize kernels, previously colonised with Foc for 10 days. In TR4-inoculated plants, first symptoms appeared at 7 days after inoculation (dai) showing the typical external (yellowing) and internal (rhizome discoloration) symptoms at 14 dai. At 40 dai, TR4-inoculated plants showed severe wilting and internal necrosis even in the pseudostem. No symptoms were observed in plants inoculated with the race 1 isolate. All the TR4 isolates caused similar symptoms, and no differences regarding incubation period or severity were observed. In addition, all TR4 isolates were successfully recovered from the symptomatic rhizomes on Komada’s medium. These results suggest that the bio-assay here described is suitable to screen banana germplasm for TR4 resistance, to discriminate Foc races and to help studies to elucidate the defence mechanism underlying the Foc-banana interaction.
Discrimination of Banana Genotypes for Fusarium Wilt Resistance in Greenhouse

L.R. Ribeiro¹, E.P. Amorim², Z.M. Cordeiro², S.O. Silva² and M.A. Dita²

¹Federal University of Reconcavo of Bahia, Cruz das Almas, 44380-000, Bahia, Brazil; ²Embrapa Cassava & Tropical Fruits, Cruz das Almas, 44380-000, Bahia, Brazil

Among the major constrains to banana breeding for Fusarium wilt resistance is the long period necessary for evaluations in the field and the lack of an effective method for early detection of resistant genotypes. This work aimed to establish a screening method for Fusarium wilt resistance under greenhouse conditions and to validate its reliability by challenging cultivars with different levels of resistance. In a first step, two types of substrates (vermiculite and washed river sand) and three inoculum sources (conidial suspension from 1-week-old colonies grown in potato dextrose agar (PDA), conidial suspension produced after stress of 1-week-old colonies and Foc-colonised corn meal-sand (CMS) medium) were studied by inoculating 45-day-old plantlets of ‘Silk’ (AAB, susceptible) in a double-tray system. Symptoms were observed in plants grown in both substrates, but highest incidence occurred in washed river sand. Low infection rates were observed when using conidial suspension from PDA-grown colonies. By contrast, inocula from stressed colonies and CMS caused consistent symptom expression. Using washed river sand as substrate and inoculum from PDA-grown stressed colonies and/or Foc-colonised CMS, plantlets of the cultivars ‘Tropical’ (AAAB) and ‘Thap Maeo’ (AAB) (field intermediate resistance) were challenged. Plantlets of ‘Silk’ and ‘Grande Naine’ (AAA) were used as susceptible and resistant controls, respectively. While the incubation period in Silk was 13 days after inoculation (dai), in ‘Tropical’ and ‘Thap Maeo’ initial symptoms were only observed at 17 dai. No symptoms were observed in ‘Grande Naine’. The disease progress evaluated based on external symptoms and rhizome discoloration scales allowed cultivars discrimination according to resistance levels. Since experiments were repeated three times with similar results, our research suggests that the method here described could be suitable for early detection of banana genotypes resistant to Fusarium wilt.
Reaction of Diploid (AA) and Tetraploid (AAAB) Banana Hybrids to Fusarium Wilt under Field Conditions

A.P. de Matos¹, Z.J.M. Cordeiro¹, S. de Oliveira e Silva¹ and D.M.V. Ferreira²

¹Embrapa Cassava & Tropical Fruits, Caixa Postal 007, 44380-000, Cruz das Almas, Bahia, Brazil; ²Agência Estadual de Defesa Agropecuária da Bahia, 44380-000, Cruz das Almas, Bahia, Brazil

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *cubense* (Foc) was first reported in Brazil in 1930 on the cultivar ‘Maçã’ (AAB, Silk subgroup). Currently, the disease is present in all banana production regions of the country. Growing resistant cultivars provides the most efficient control of Fusarium wilt. In this work, seven diploid (AA) and fourteen tetraploid (AAAB) banana hybrids were evaluated for resistance to Foc. Trials were conducted under field conditions on infested soil, from where Foc isolates were classified as belonging predominantly to VCGs 0124 and 0125. ‘Maçã’ and ‘Figue Pome Naine’ (AAB, Silk type) were used as susceptible controls. All diploid hybrids, namely ‘011601’, ‘130404’, ‘130406’, ‘131801’, ‘422306’, ‘51A1901’ and ‘SH3263’, showed resistance to the pathogen during both first and second crop cycle. Among the fourteen tetraploid hybrids evaluated during two crop cycles, ‘PV42-53’, ‘PV42-68’, ‘PV42-81’, ‘PV 42-85’, ‘PV42-142’, ‘PV42-143’, ‘ST42-08’, ‘ST12-31’, ‘PV03-44’, ‘FHIA-03’ and ‘SH36-40’ expressed resistance to Fusarium wilt, while ‘PV42-129’, ‘PC42-01’ and ‘YB 42-21’ behaved as susceptible. Results also showed that seven out of ten hybrids obtained from crossings in which ‘M53’ (AA) was used as male parental showed resistance to Foc, thus suggesting that ‘M53’ is a promising parental to be used in banana breeding programmes aiming at developing Fusarium wilt-resistant cultivars.

Fusarium Wilt Management through Use of Resistant Genotypes, Chemical and Biological Approaches

A. Cherian, P.M. Mathew, R. Menon, A. Suma, K.C. Aipe and S.N. Nair

*Banana Research Station, Kerala Agricultural University, Kannara P.O, Thrissur, Kerala, India*

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* is a serious fungal disease of banana, affecting commercial cultivars in India like ‘Rasthali’
(AAB), ‘Njalipoovan’ (AAB) and ‘Kadali’ (AAB). In order to identify potential sources of resistance, the 296 accessions of the field genebank maintained at the Banana Research Station (BRS), Kannara were screened for the natural occurrence of the disease. The most promising cultivars were raised in pots and artificially inoculated to confirm resistance. Banana hybrids ‘BRS I’ and ‘BRS II’ released by BRS were found to be highly resistant. ‘Nendran’ (AAB, French plantain), the most widely grown cultivar, was also found to be resistant. The disease could be effectively managed by drenching the soil with carbendazium at 0.2% at 5, 7 and 9 months after planting. The ecofriendly biocontrol agents *Pseudomonas fluorescens* and *Trichoderma viride* also proved to be effective for integrated management of Fusarium wilt and of the Fusarium-nematode complex in banana.

**Selection of a New Somaclone Cultivar ‘Tai-Chiao No.5’ with Resistance to Fusarium Wilt of Banana in Taiwan**


Taiwan Banana Research Institute, Pingtung, Taiwan, R.O.C

The exploitation of somaclonal variation is an efficient approach to select Cavendish-type banana cultivars with superior horticultural traits and resistance to Fusarium wilt, caused by *Fusarium oxysporum* f. sp *cubense* race 4 (Foc race 4, VCG1213/1216), in Taiwan. ‘TC3-1035’, an improved somaclonal variant of ‘Tai-Chiao No.3’ was selected and released as a new banana cultivar ‘Tai-Chiao No. 5’ in 2007. It inherited the semi-dwarf status and moderate resistance to Foc race 4 of ‘Tai-Chiao No.3’, but not the susceptibility to flower thrips, *Thrips hawaiiensis*, at shooting stage. It also has a higher yield potential. Under suitable field management, plant height of ‘Tai-Chiao No. 5’ is 2.7 m, and its fruit size, shape and yield capacity are as good as those of ‘Pei-Chiao’, which is the most widely planted Cavendish cultivar in Taiwan. During the 7-year consecutive evaluation trials, Fusarium wilt incidence in ‘Tai-Chiao No. 5’ plants was 5-25%, whereas 10-50% plants were affected in ‘Pei-Chiao’ established in the same infested plot. This is an indication of the stable resistance of ‘Tai-Chiao No. 5’ to Foc race 4. With additional benefits of easy ripening and good eating quality, ‘Tai-Chiao No. 5’ has now been well accepted both in the local and the Japanese market. Currently, a total of 0.9
million tissue-culture plantlets, about 450 ha, have been released to banana growers in Taiwan.

**Comparison of Host Reaction to *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 and Agronomic Performance of Somaclonal Variant ‘GCTCV 119’ and ‘Grand Naine’ in Commercial Farms in the Philippines**

E.T. Fabregar¹, R.O. Soquita¹, V.S. Sinohin² and A.B. Molina²

¹Lapanday Fruits and Development Corp., Davao City, Philippines; ²Bioversity International - Commodities for Livelihoods programme, c/o IRRI, Los Banos, Laguna 4031, Philippines

Recent reports of the occurrence of *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (Foc TR4), causal agent of Fusarium wilt, in some commercial Cavendish banana plantations in the Philippines is of great concern to the Philippine banana industry, which ranks second in global banana export after Ecuador. In an effort to mitigate the disease, ‘GCTCV 119’, a Foc TR4-resistant somaclonal variant of ‘Giant Cavendish’ selected by the Taiwan Banana Research Institute, was evaluated and compared with the commercial cultivar ‘Grand Naine’ in Foc-infested farms in Davao, Philippines. Samples of infected tissues from these farms were previously analysed positive for VCG1213/16, the vegetative compatibility group responsible for TR4. Tissue culture-derived planting materials of ‘GCTCV 119’ and ‘Grand Naine’ were planted in randomised blocks in two farms where severe wilt incidence was previously observed. After 12 months of disease incidence monitoring, ‘GCTCV 119’ showed no Fusarium infection, while 36 and 51% incidence was recorded for ‘Grand Naine’ in Calinan and Quibilan farms respectively. Two years after establishment, ‘GCTCV 119’ plots in the Quibilan farm are still free from infection of Fusarium wilt while the ‘Grand Naine’ plots have been totally eradicated because of severe disease incidence. Agronomic and fruit quality traits were also recorded. While ‘GCTCV 119’ proved to be highly resistant to Fusarium wilt TR4, it matured later and had a lower bunch weight and greater hand curvature resulting in more rejects when packed. ‘GCTCV 119’ is however sweeter as it has more total sugars and total soluble solids than ‘Grand Naine’.
Combating Banana Wilts – What Do Resistant Cultivars Have to Offer?

J.W. Daniells

Queensland Primary Industries & Fisheries, South Johnstone, Queensland, Australia

Fungal and bacterial wilts of banana are currently rampaging through many parts of Asia and Africa, greatly reducing the profitability of production and impacting very significantly on food security. Fusarium wilt and the “bacterial trinity” of Moko/bugtok, blood bacterial wilt and Xanthomonas wilt are causing major problems to both commercial and subsistence plantings. Most recently, tropical race 4 of Fusarium wilt has destroyed in excess of 20,000 ha of Cavendish bananas in China and threatens the huge export industry of the Philippines. Resistant cultivars are often touted as the solution to banana disease problems, including the wilts. However, resistant cultivars must be part of an integrated disease management programme to maximise the chances of their deployment being successful in combating banana wilts. Experience has also shown that replacing susceptible cultivars is far from a simple matter in gaining acceptance in the marketplace and that cultivars resistant to wilts will likely have their own set of pest and disease problems. Cultivars with known resistance to Fusarium wilt and those with likely resistance to bacterial wilts based on bunch morphology are presented along with best-bet management procedures for good results.

Fusarium Wilt Incidence, Growth, Yield and Post-Harvest Quality of Banana as Affected by Organic Farming System in Taiwan

C.M. Chang¹, C.P. Chao¹, S.N. Huang¹ and S.C. Chiang²

¹Taiwan Banana Research Institute, Chiuju, Pingtung, Taiwan, R.O.C; ²Taiwan Environmental Organics Application Association, Chiuju, Pingtung, Taiwan, R.O.C

Organic banana farming at TBRI, characterised by chemical-free management practices, is an alternative to conventional banana production management in Taiwan. Soybean cake and palm ash are chosen as the nutrient source of
nitrogen and potassium for the banana plants. Enhanced sanitation and regular spraying of foliage and pseudostem with an extract of mixed hot pepper and garlic solution are applied to control diseases and pests. Results from a 10-year study on organic banana production at the TBRI organic farm indicate that average incidence of Fusarium wilt for four Cavendish cultivars was 25.7% for the organic farming system, as compared to 66.9% for the conventional farming system. This resulted in a higher overall yield in the organic farming system. The lower incidence of Fusarium wilt in the organic banana was considered to be associated with a positive change in the physico-chemical and biological properties of the soil. Horticultural parameters at shooting stage, such as plant height, pseudostem girth, number of hands per bunch and number of fingers per hand, did not show significant differences between organic and conventional banana, except for a lower number of healthy leaves in the organic banana. A smaller bunch weight was also recorded for the organic banana; however, no differences in the post-harvest quality could be found between organic and conventional bananas. In summary, the organic farming system adopted in the TBRI farm offered the following advantages over the conventional farming system: (a) The incidence of Fusarium wilt was markedly lower, and subsequently the yield and income were significantly increased; (b) The soil quality of the organic banana plantation was positively affected, indicating a restoration of soil productivity in the organic plantation; and (c) Banana production adopting organic farming practices allowed producing bananas of the same quality as bananas produced under the conventional system. Promotion of the organic farming system for adoption by more banana growers could increase the sustainability of the Taiwan banana industry.

**Efficacy of Clay-Based Formulated Serratia in Reducing Inoculum of Fusarium oxysporum f. sp. cubense Race 4**

A.S.Y. Ting, M.T. Fang and C.S. Tee

*Faculty of Engineering and Science, Universiti Tunku Abdul Rahman, Kuala Lumpur, Malaysia*

The bacterial isolate *Serratia*, isolated from roots of wild banana, has shown beneficial properties in previous studies when associated with banana plants, promoting plant growth in plantlets at both glasshouse and field stage, and
suppressing Fusarium incidence, albeit only at the glasshouse stage. The loss of control efficacy in the field was attributed to low inoculum levels of the bacterium in the soil. Therefore, this study aimed to develop a suitable formulation for the isolate to enhance its viability and efficacy in field applications. A comparison between sunlight-exposed and non-exposed formulations was also made. The clay-based materials bentonite and kaolin were selected as carrier materials as they are naturally abundant in the environment. To each clay, non-fat skimmed milk (NFSM) and sucrose were added as enrichment materials, and para-aminobenzoic acid (PABA) was added for its assumed UV-protectant properties, in various combinations. Preliminary laboratory experimental results revealed that formulation with bentonite was slightly more efficient in reducing the inoculum level of *Fusarium oxysporum* f. sp. *cubense* race 4 (Foc R4) compared with kaolin, with $4.06 \log_{10} \text{CFU/ml}$ and $4.28 \log_{10} \text{CFU/ml}$, respectively. This was attributed to the higher viable cell count of *Serratia* recovered, especially from formulations incorporated with NFSM and sucrose. Bentonite formulations also produced higher viable cell count for both sunlight-exposed and non-exposed treatments. PABA on the contrary was discovered to have antimicrobial properties towards both *Serratia* and Foc R4. The UV-protectant effect was also absent in all formulations incorporated with PABA resulting in low viable cell counts of *Serratia*. Therefore, for future applications, it is suggested to omit PABA from the bentonite formulation, while NFSM and sucrose levels can be further optimised for economical reasons. This biotechnological formulation is also highly cost-effective as the total cost for formulations with combinations of bentonite, NFSM and sucrose was less than USD10 for every 100 g.

**Genetic Structure of *Mycosphaerella fijiensis* Populations in Costa Rica**

S.A.L. Garcia$^{1,4}$, M. Guzmán$^2$, E.C.P. Verstappen$^1$, T.A.J van der Lee$^1$, S.B. Goodwin$^3$, M.T. Souza Jr$^{1,5}$ and G.H.J. Kema$^1$

$^1$Plant Research International, 6708 PB, Wageningen, The Netherlands; $^2$Corbana, PO Box 6504-1000 San José, Costa Rica; $^3$USDA-ARS, Purdue University, 915 W. State Street, West Lafayette, IN, 47907-2054, USA; $^4$Universidade Federal de Lavras, Caixa Postal 3037, Lavras- MG, Brazil; $^5$Embrapa LABEX Europe, 6708 PB, Wageningen, The Netherlands

*Mycosphaerella fijiensis* is the causal agent of black leaf streak of banana, the most important threat to banana production in many countries and particularly
in Costa Rica where the climate is very conducive for the disease. Currently, the main control measure is the frequent application of fungicides. However, apart from environmental concerns, this approach is not sustainable due to the abrupt or gradual development of fungicide resistance. To analyse the population dynamics of fungicide resistance, we developed molecular diagnostics for strobilurin resistance, using the cytochrome b gene (cytb), in *M. fijiensis*. We also developed molecular markers for the mating type idiomorphs (*mat1-1* and *mat1-2*) and primers for five VNTR loci to estimate population genetic parameters. Monospore isolates were collected at three plantations that are 20-30 km apart (Cartagena, San Pablo and Zent) in the Limón province that represents the heart of the Costa Rican banana production area. Ninety-five (95) isolates were obtained from a distant wild-type population that was never sprayed with fungicides in the Heredia province. In total, 665 isolates were assayed for *mat1-1*, *mat1-2*, VNTR and *cytb*. The mating type genes segregated in a 1:1 ratio indicating that the sampled populations most likely are randomly mating. The strobilurin diagnostic indicated that the wild-type population is entirely sensitive and that two of the three commercial populations are entirely resistant. The Zent population contained 8% of sensitive strains, even though strobilurins were still used in that plantation. The VNTR primers identified 33 alleles and high levels of gene diversity within each population (*h* = 0.402-0.487). Analysis of molecular variance revealed that 92% of the total variation was within populations with only 8% due to differentiation among them. Levels of geneflow were high (*Nm* = 4-14 individuals per population pair per year) but populations were still slightly but significantly different. These analyses provide an excellent basis for future research into fungicide resistance in Costa Rican populations of *M. fijiensis* as well as comparative analyses with other banana-producing areas.
Transcriptome Analysis of *Mycosphaerella fijiensis*, the Causal Agent of Black Leaf Streak Disease in Banana

C. Diaz-Trujillo\(^1,2\) , B. Te Lintel Hekkert\(^1\) , E.A. Lindquist\(^3\) , J. Carlier\(^4\), M.T. Souza Jr\(^1,5\) and G.H.J. Kema\(^1\)

\(^1\)Plant Research International, PO Box 16, 6700 AA Wageningen, The Netherlands; \(^2\)Wageningen University Graduate School of Experimental Plant Sciences, PO Box 16, 6700 AA Wageningen, The Netherlands; \(^3\)DOE - Joint Genome Institute, 2800 Mitchell Drive, Walnut Creek, CA 94598, USA; \(^4\)Biologie et Génétique des Interactions Plantes-Parasites, CIRAD-INRA-AGRO.M, TA 41/K, 34398 Montpellier, France; \(^5\)Embrapa LABEX Europe, PO Box 16, 6700 AA Wageningen, The Netherlands

*Mycosphaerella fijiensis* is the causal agent of black leaf streak disease (also known as black Sigatoka), the most devastating fungal disease of banana worldwide. Control of the disease requires excessive fungicide applications to prevent effects on photosynthesis resulting in premature ripening of the fruit. Because the biology of this pathogen is still poorly understood, genomic and molecular tools are being developed. The whole-genome sequencing of *M. fijiensis* isolate CIRAD 086 (Cameroon) was concluded at the Joint Genome Institute (JGI-DOE), and initial analyses indicate a genome size of 73.4 Mb, which is almost twice the genome of the closely related *Musa graminicola* and is the largest genome currently available of an ascomycete fungus. A transcriptome project was carried out via the production and sequencing of three cDNA libraries from *M. fijiensis* CIRAD 086 grown in different in-vitro conditions (yeast glucose, minimal medium with nitrogen, minimal medium without nitrogen), and the results are described here. Forward and reverse sequences were produced and trimmed for vector and quality, resulting in 32,392 ESTs. All together, this set of ESTs encompasses approximately 22.5 Mb of high-quality sequence, with a 53.25% GC content. After the assembling, a set of 12 genes, selected among the most populated contigs from each of the different cDNA libraries combinations, as well as orthologs genes of known function in other pathogens, were selected for expression studies. These studies are being conducted by RT-PCR analysis of samples collected on both in-vitro and in-vivo conditions. This transcriptome database is by far the most complete analysis of the expressed genes in this pathogen and an important resource to help on whole-genome assembly and gene discovery in *M. fijiensis*. 
Bridging the Technology Gap in Banana Improvement: The Case of Biotechnology Adoption by Developing Countries

M.N. Mwangi

Department of Biochemistry and Biotechnology, Kenyatta University

Gamma irradiation and ethyl methane sulphonate (EMS) were used to induce mutants of two popular Kenyan dessert cultivars ‘Kampala’ (AAA) and ‘Nyoro’ (AAA) in an attempt to improve their resistance to black leaf streak, caused by *Mycosphaerella fijiensis*, and Sigatoka leaf spot, caused by *Mycosphaerella musicola*. Intact and longitudinally dissected shoot tips of in-vitro cultures of the two cultivars were exposed to 11 doses of $^{60}$Co gamma (ranging from 0-100 Gy) at a dose rate of 8 min$^{-1}$ and 4 concentrations (25, 50, 75, 100 mM) of EMS. The mutagen-treated shoot tips were regenerated into plantlets and screened for host response, both in vivo and in vitro using standard methods. The incubation and evolution phases of the diseases were significantly different between the mutagen-treated and the untreated controls. For the more virulent *M. fijiensis* pathogen, a second double in-vitro selection procedure, involving the use of crude fungal filtrate followed by the purified toxin, was conducted. Compared with the non-treated control plantlets, 12-20% of the toxin-tolerant plants were resistant to *M. fijiensis* but some undesirable variations appeared. Forty-five percent of the resistant ‘Nyoro’ plants were late flowering, small in stature (<1 m), with thick pseudostems (40-45 cm in circumference), short internodes and deformed leaves. They also produced smaller bunches with a 25-75% reduction in number and size of fingers. Among the mutagen treated ‘Kampala’ plants, 5% were significantly taller than the control plants, with long internodes and large fruit. Over two production cycles, the control plants were generally more susceptible to black leaf streak and Sigatoka leaf spot but had superior agronomic traits (earliness, high suckering, large leaf area and bunch size). The results are discussed with respect to the application of in-vitro mutation induction as a strategy to bridge the biotechnology adoption gap by developing countries.
Characterisation of the Digestive Proteases in the Banana Weevil (Cosmopolites sordidus) Gut and the Effects of Recombinant Phytocystatins and Bt Cry6A on Early Larval Growth and Development

A. Kiggundu¹, K. Kunert², D. Michaud³, A. Viljoen⁴, W. Tushemereirwe⁵ and E. Karamura⁶

¹National Agricultural Research Organisation, National Agricultural Research Laboratories Institute, PO Box 7065, Kampala, Uganda; ²Forestry and Agricultural Biotechnology Institute, Department of Plant Science, University of Pretoria, 74 Lunnor Road, Hillcrest, Pretoria 0002, South Africa; ³Département de Phytologie, Pavillon Paul-Comtois, Université Laval, Sainte-Foy (Québec), Canada G1K 7P4; ⁴Institute for Plant Pathology, Stellenbosch University, Matieland 7602, South Africa; ⁵National Agricultural Research Organisation, National Crops Resources Research Institute, Namulonge, PO Box 7084, Kampala, Uganda; ⁶Bioversity International, PO Box 24384, Kampala, Uganda

The potential of developing insect-resistant transgenic plants has expanded, and several insecticidal proteins such as proteinase inhibitors have been proposed for the control of crop pests. Insects possess different protease forms used to digest dietary proteins, and studies to characterise the forms of protease are important to provide the basis for selecting appropriate protease inhibitors likely to be effective in a transgenic approach. In this study, the protease activity in the gut of banana weevil Cosmopolites sordidus was analysed in order to determine the potential of using phytocystatins for the control of the banana weevil. Extracts from larvae guts were found to hydrolyse casein at an acidic pH optimum (pH 5.5). Lesser activity was also detected at alkaline pH conditions (pH 8.0). Cathepsin L- and B-like cysteine proteases were most abundant in the larval gut, as shown by hydrolysis of the specific substrates Z-Phe-Arg-MCA and Z-Arg-Arg-MCA, respectively. Much smaller amounts of trypsin and chymotrypsin-like serine proteases were also detected using the specific substrates Bz-Arg-MCA and N-Suc-Ala-Ala-Pro-Phe-MCA, respectively. Oryza cystatin-I and corn cystatin-II inhibited the weevil cysteine protease activity whereas a soybean trypsin-chymotrypsin inhibitor inhibited serine protease activity. Recombinant oryza cystatin-I and papaya cystatin inhibited cysteine protease activity in the banana weevil gut homogenates by
66.2 and 81.6% and LD<sub>50</sub> of 1x10<sup>-5</sup>ng/ml and 2.1x10<sup>-5</sup>ng/ml, respectively. Using novel in-vivo assays including artificial diets weight gain per day of larvae was inhibited by 77% at an inhibitor concentration of 0.6 mg of cystatin/g fresh weight in larvae that developed on cystatin-treated stem disks. The study therefore confirms cysteine proteases similar to cathepsin L and B are the major modes of protein digestion in banana weevil, and their inhibition can form part of a control approach. Finally, recent developments in assessing the effect of cystatin and Bt Cry6A combinations on weevil are presented and the potential of a gene combination approach discussed.

Screening 40 Musa Genotypes for Banana Bunchy Top Virus Resistance in Burundi

C. Niyongere<sup>1</sup>, E. Miinda Ateka<sup>2</sup>, T. Losenge<sup>2</sup>, P. Lepoint<sup>3</sup> and G. Blomme<sup>4</sup>

<sup>1</sup>ISABU, PO Box 795, Bujumbura, Burundi;  <sup>2</sup>Department of Horticulture, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya;  <sup>3</sup>Bioversity International, Bujumbura, Burundi;  <sup>4</sup>Bioversity International, Uganda office, PO Box 24384, Kampala, Uganda

Bunchy top disease, caused by the *Banana bunchy top virus* (BBTV), is one of the most devastating diseases affecting banana and plantain cultivation. In order to find sources of resistance, a variety screening trial comprising 40 *Musa* genotypes was established in March 2007 at the ISABU Mparambo research station in north-western Burundi (S2°50'220; E29°04'375; altitude of 893 masl). Dessert bananas (AAA genome group), East African highland bananas (AAA, EAHB), plantains (AAB), cooking bananas (ABB), tetraploid hybrids and wild diploid bananas (*Musa acuminata* and *Musa balbisiana*) are being assessed. Ten plants per genotype were planted out in a completely randomised design. Border rows of BBTV-infected ‘Yangambi km5’ (AAA) plants were planted in between lines. Aphids (*Pentalonia nigronervosa* – the BBTV vector) collected in diseased farmers fields were also released in the plot. Two years after trial establishment, 32 accessions have shown BBTV disease symptoms. The first disease symptoms appeared 4 months after trial establishment on ‘Poyo-Cavendish’ (AAA) and ‘Chibulangombe’ (AAA, EAHB). Currently only eight genotypes, namely *Musa balbisiana* type ‘Tani’, ‘Fougamou’ (ABB), ‘Cacambou’ (ABB), ‘Gisandugu’ (ABB), ‘Pisang Awak’ (ABB), ‘Saba’
(ABB); ‘Prata’ (AAB) and ‘Highgate’ (AAA), have not shown any visible disease symptom on any of the 10 plants per genotype. However, plant samples taken from these visibly healthy genotypes and analysed at the Gembloux University in Belgium indicated the presence of the virus in ‘Pisang Awak’, ‘Saba’, ‘Highgate’ and ‘Cacambou’. These cultivars can be considered tolerant and could act as a reservoir of the virus. Preliminary results show that genotypes with one or two B genomes are more tolerant to BBTV.

Banana Bunchy Top Virus-Resistant Transgenic Banana Plants

W. Borth¹, E. Perez¹, K. Cheah², Y. Chen¹, W.S. Xie¹, D. Gaskill¹, S. Khalil¹ and J.S. Hu¹

¹Plant and Environmental Protection Sciences, University of Hawaii at Manoa, Honolulu, HI, USA; ²Tropical Plant and Soils Sciences, University of Hawaii at Manoa, Honolulu, HI, USA

Embryogenic cell suspensions (ECS) initiated from immature male flowers of banana cultivar ‘Dwarf Brazilian’ (AAB) were transformed using Agrobacterium tumefaciens containing one of four constructs derived from the replicase-associated protein (Rep) gene of the Hawaiian isolate of Banana bunchy top virus (BBTV). Each construct was engineered under control of the CaMV 35S promoter in the binary plasmid pBI121. Constructs were transferred into A. tumefaciens strain AGL0 and used to transform banana ECS. Plantlets that survived antibiotic selection were acclimatised to greenhouse conditions and challenged with viruliferous banana aphids (Pentalonia nigronervosa). Ten adult or late instar aphids were allowed to feed for 2-4 weeks on test plants. All test plants were kept in the greenhouse and monitored for symptom expression for a period of 6 months. Control plants transformed with empty vector pBI121 only were included in all tests. A total of 270 test plants and 63 control plants were screened for BBTV resistance using this approach. One of 32 test plants transformed with the M1 (mutant Rep gene) construct, 4 of 74 test plants transformed with the AS (antisense Rep gene) construct, 5 of 38 test plants transformed with the Δ2/5 (partial Rep gene) construct, and 10 of 126 test plants transformed with the RΔ2/5 (inverted repeat of partial Rep gene) construct were found to be resistant/tolerant to BBTV and showed no bunchy-
top symptoms. All of the control plants became infected with BBTV under these experimental conditions. Plants that survived BBTV challenge were analysed by quantitative PCR (qPCR) and Southern hybridisations to determine the number of transgenes that were present in their genomes. Results from these analyses indicated that the resistant plants contained from 2 to more than 9 copies of the NPTII (kanamycin resistance) transgene carried on the pBI121 plasmid.

**Management of Banana Diseases and Pests by Use of Tissue Culture-Derived Planting Material in Kenya**

J. Njuguna, S. Nguthi, F. Wambugu, D. Gitau and M. Karuoya

*Horticultural Research Centre, Kenya Agricultural Research Institute, National Horticultural Research Centre, PO Box 220, 01000, Thika, Kenya*

Banana is a popular food crop in Kenya. Around 2 million tonnes are produced annually from about 80,000 ha. However, production has been declining in recent years, mainly due to diseases and pests. Farmers traditionally use suckers from their old orchards or from their neighbours’ fields for replanting. These materials are often infected by pathogens, particularly *Fusarium oxysporum* f. sp. *cubense*, weevils and nematodes. This practice has led to the spread of diseases and pests to many production areas leading to declining productivity. In 1997, the Kenya Agricultural Research Institute started distributing tissue-cultured banana plantlets to farmers’ groups. A participatory evaluation approach was used for farmers to compare the performance of their traditional materials and tissue-cultured materials. Key consideration was the economic benefits of using tissue-culture materials as a way of disease management and increasing productivity. The project initially worked with eight groups of 500 farmers in Eastern and Central Provinces of Kenya. The number of groups later increased to 17 with a total of 966 members. Farmers were trained on all aspects of tissue-culture production using the farmer field school approach. By 2001, about 2,500 farmers were growing about 107,600 tissue-cultured banana plants. By adopting the tissue-culture technology, the yield increased from less than 10 t/ha to about 30 t/ha. It was found that tissue-cultured materials were free of diseases and pests for a longer time than the traditional suckers. It was also found that, although tissue-cultured materials were more expensive than
the traditional suckers, the extra cost was more than offset by the increased yield. It can therefore be concluded that tissue-culture technology has a great potential for managing banana diseases and pests in an integrated approach system.

**Virus-Indexing Technology in Banana: A Boon to the Tissue-Culture Industries and Banana Growers for Production of Quality Planting Material in India**

R. Selvarajan, V. Balasubramanian, M. Mary Sheeba, R. Raj Mohan and M.M. Mustaffa

*National Research Centre for Banana (NRCB-ICAR), Thogamalai Road, Thayanur (Post), Tiruchirapalli 620102, Tamil Nadu, India*

Banana and plantain are the most important fruit crop in India and play a major role in the livelihood of millions of resource-poor small farmers. Though conventional suckers are still the main planting material used for cultivation, use of tissue-culture plants has increased because of their advantages, like more uniform bunches with even maturity and increased yield. However, banana viral pathogens, which are economically significant in India, can be inadvertently spread through tissue-culture plants. In order to control the spread of the viruses, efforts were taken at the National Research Centre for Banana, Tiruchirapalli to develop virus-indexing techniques for early detection in mother plants and tissue-culture plants used for mass propagation. PCR, Reverse transcriptase PCR (RT-PCR), non-radioactive probe-based Nucleic Acid Spot Hybridisation (NASH) and ELISA-based techniques were developed and validated for routine testing. PCR and NASH tests are being done for detection of *Banana bunchy top virus* (BBTV) and *Banana streak Mysore virus* (BSMyV). RT-PCR and ELISA tests are being done for the detection of *Banana bract mosaic virus* (BBrMV) and *Cucumber mosaic virus* (CMV). Based on the work done at the centre, the Department of Biotechnology (DBT), Government of India, has accredited the Molecular Virology lab for testing for banana viruses in the country. In total, 15,850 tissue-culture and mother-plant samples were tested against viruses. The percentage of positive plants for BBTV, BSMyV, CMV and BBrMV were 3.83%, 2.85%, 17.3% and 0.95%, respectively.
respectively. Indexing was done mostly for the cultivars ‘Grande Naine’ (AAA, Cavendish subgroup), ‘Robusta’ (AAA, Cavendish subgroup) and ‘Hill Banana’ (syn. ‘Virupakshi’, AAB, Pome subgroup). Around 25 tissue-culture banana industries are undertaking the services of virus testing. Our laboratory has also developed polyclonal antisera for CMV and BBrMV through recombinant DNA technology, which is currently being validated for ELISA-based testing. The future requirement of virus-indexing kits for tissue-culture banana and the utility of the kits in quarantine, breeding programmes and direct use by farmers are discussed in detail.

Opening of Workshop 2: Fusarium wilt – A Banana Disease that Refuses to Go Away

R. Ploetz¹, A. Churchill² and A. Viljoen³

¹University of Florida, 18905 SW 280th Street, Homestead, Florida 33031-3314, USA; ²Cornell University, Ithaca, NY, USA; ³Institute for Plant Pathology, Stellenbosch University, Matieland 7602, South Africa

Fusarium wilt (also known as Panama disease) is the most important lethal disease of banana. The causal soilborne fungus, Fusarium oxysporum f. sp. cubense, is found in most banana-producing regions, and is phylogenetically and pathogenically diverse. Races 1 and 2 are heterogeneous and include numerous vegetative compatibility groups (VCGs), while those that affect the Cavendish subgroup are fairly well defined; they are separated into subtropical race 4 (SR4) (VCG 0120 and related VCGs) and tropical race 4 (TR4) (VCG 01213-01216 complex). SR4 affects Cavendish plants that have been predisposed to disease by cold temperatures in the subtropics, whereas TR4 kills Cavendish under tropical conditions in the absence of predisposing factors. Race 1 devastated export plantations of ‘Gros Michel’ prior to the mid-1900s. Fusarium wilt diminished in importance as the trades converted to the race 1-resistant Cavendish subgroup, but it has re-emerged as a major threat due to the impact and spread of TR4. TR4 now decimates Cavendish monocultures in southern Asia and could well affect 85% of global banana production if it were disseminated more widely. Measures are needed to diagnose, interdict and effectively manage TR4, as well as other variants worldwide. This session will be introduced with an historical perspective on Fusarium wilt of banana,
Oral Presentations - Session 2

followed by synopses of recent work on the pathogen, host and disease management. Updates for countries and regions will be presented, and current work of individuals and research groups will be summarised. The session will close with a roundtable discussion on key issues and data gaps that should be addressed. Although future work will be focused by funding constraints, mutually beneficial objectives will facilitate intergroup cooperation on this serious disease.
Closing Session

Closing Keynote 1: Harnessing the Potential of Banana and Plantain in Asia and the Pacific for Inclusive Growth

H.P. Singh

Indian Council of Agricultural Research, New Delhi, India

Banana and plantain was one of the earliest crops domesticated by man and it continues to be an important food crop for millions of smallholders as well as an important high-value crop in international trade, providing nutritional and economic security. It is grown in around 150 countries across the world, with around 97 million tonnes produced annually on an area of 6.25 million hectares. Asia contributes 55-60% of the global production, and has in the last 6 years recorded a production growth rate of 11%. India, the largest producer of banana in the world, witnessed unprecedented growth during this period, due to improved production technologies, which include adoption of high-yielding clones, high-density planting, integrated management of water, nutrients, pests and diseases, and use of disease-free, in-vitro propagated planting material. Similar is the case in other Asian countries, with Taiwan and the Philippines producing mainly for export through contract farming systems and the other Asian countries producing mainly for domestic consumption and trade by small farmers. BAPNET, the regional banana and plantain network for Asia and the Pacific, is fostering collaborative banana research among its 13 member countries and two research institutes. Looking at the strengths, weaknesses and opportunities for banana in Asia and the Pacific, it is evident that the region has a rich diversity of *Musa* which needs exploitation. The main thrust of the network has indeed been collection, conservation, evaluation and utilisation of banana diversity, testing of new hybrids, exchange of germplasm and capacity building. At the same time, biotic and abiotic stresses are many. Biotic problems include viruses, fungal diseases and insect pests, while abiotic
stresses include drought, salinity and various vagaries of climates. Fusarium wilt is but one example of a devastating disease, with no transboundary restrictions. Race 4 of Fusarium wilt has already spread to China, Taiwan, Malaysia and Australia. In this regard, BAPNET and its member countries are drawing management strategies, including internal and transboundary quarantine, sanitation, early detection, improving soil health, biological control and altered crop production systems. Production of disease-free tissue-culture planting material and diagnostics, as well as the establishment of a certification system in some of the countries, have been milestone achievements. International initiatives on genetic resources, management of diseases and insect pests, water productivity and nutrition, post-harvest management and processing remain crucially important to ensure nutritional and livelihood security and enhance the income of smallholders. Efforts of Bioversity International have, through various programmes, improved the understanding of genomics, diversity, biotic and abiotic stresses and augmentation of genetic resources for gene transfer. The horizontal and vertical integration with National Agricultural Research Systems allows to better address the many problems of smallholder banana farmers. The keynote address touches upon the emerging issues and identifies the area of cooperation.

Closing Keynote 2: Making Science Relevant - Linking Agricultural Research Networks to Innovation Platforms

S. Weise

Bioversity International, Parc Scientifique Agropolis II, 34397 Montpellier Cedex 5, France

Agricultural research networks have played an important role in bringing together researchers and research institutions. This has allowed the sharing of knowledge, discussion of new findings, identification of priorities, promotion of collaborative activities and exploitation of synergies. Nevertheless, within a research-for-development framework, the link of such networks to agricultural development efforts and strategies, particularly in a small-holder farming environment, has been and is often weak. Innovation platforms on the other hand bring together key stakeholders, for example across a commodity value chain. The goal is to jointly address key production, marketing, institutional
and policy constraints and opportunities towards economic and sustainable growth in a subsector. Key to the success of such a platform of public and private sector partners is the development of a common vision of engagement to the benefit of all the key value chain stakeholders. Research plays a key role in identifying options and analysing outcomes across the value chain. At the same time, the innovation platform provides a ‘reality-check’ for research – from prioritisation through to implementation and evaluation – by assuring issues are addressed as identified by stakeholders who have direct vested interest in a commodity value chain. It is thus postulated that research networks, to be effective, need to be associated with innovation platforms.
Poster Presentations
Musa Collection, Characterisation and Improvement in China


Fruit Tree Research Institute, Guangdong Academy of Agricultural Sciences, Guangdong Province, China

Banana is an important crop in China. Musa germplasm collection was started in China in the 1950s and initially focused on some local cultivars. The National Field Genebank for Banana (Guangzhou) was built in 1989, with around 170 local and exotic accessions collected until then. Another 32 accessions were introduced in 2002 and another 33 accessions in 2007 from the International Transit Centre (ITC, Belgium). The collection currently holds 291 accessions. During collection missions in southern China (mainly Guangdong, Guangxi, Hainan, Yunnan, Fujian and Hunan provinces), 178 domestic accessions were collected, including 34 wild accessions belonging to 6 species, 70 Cavendish (AAA) accessions, 33 Dajiao (ABB) accessions and some other types. Descriptors and Data Standards for Banana were published in Chinese in 2006, and most of the germplasm was characterised. A Chinese Musa Germplasm Information System (CMGIS) database was set up. Evaluation of the accessions indicated that some cultivars are resistant to Fusarium oxysporum f. sp. cubense (Foc) race 4, namely ‘FHIA-01’ (AAAB), ‘FHIA-25’ (AAAA), ‘Inarnible’ (AA), ‘GCTCV-119’ (AAA), ‘Xiao Mi Jiao’ (ABB), and some local Dajiao (ABB) cultivars. Germplasm improvement through screening and selection of tissue-cultured plants is ongoing, and some new cultivars have been obtained, especially of the Cavendish type, such as ‘Dafeng
No.1’, ‘Dafeng No.2’ and ‘Bisheng’. They are currently used in banana production. Cross breeding also was done, and a new cultivar ‘Fengzha No.1’ with resistance Foc race 4 was developed.

**Banana Germplasm Investigation, Collection and Conservation in Guangxi, China**

J.Y. Yao¹, X.Q. Qin², X. Long¹, H.X. Peng¹

¹Horticultural Research Institute, Guangxi Academy of Agricultural Sciences, Nanning 530007, China; ²Agricultural College, Guangxi University, Nanning 530005, China

Guangxi province in China lies in the centre of origin of banana, and plenty of wild banana species and cultivars have grown there for centuries. Field-based investigation and conservation of this germplasm will provide valuable genes or characters for banana resistance breeding and genetic modification. A general investigation and collection of wild and cultivated germplasm in Guangxi Zhuang Autonomous Region showed a wide distribution of wild banana species, which ranges from Ziyuan county in the north to Fangchenggang and Dongxing city in the south, and from Leye and Tiane county in the west to Wuzhou in the east. The main distribution can be found in the north-west and south-west of Guangxi, where very high mountains with dense vegetation are located. Germplasm collected from different areas showed great differences in buds, fruits and plants indicating their genetic diversity. Based on 25 morphological characters, wild germplasm which set fruits filled with seeds were classified into Musa, Callimusa and Australimusa, and were identified as five species: *Musa acuminata*, *Musa itinerans*, *Musa schizocarpa*, *Musa coccinea* and *Musa peekelia*. All collected banana germplasm are conserved by seeds, suckers/plants or pollen at the Horticultural Research Institute of Guangxi Academy of Agricultural Sciences (GXAAS). Further characterisation and molecular phylogeny of this germplasm is required to assess their genetic diversity and their potential value for banana breeding.
Preliminary Study on ISSR Analysis and Classification of Wild Banana \((Musa\ spp.)\) in Guangxi, China

X.Q. Qin\textsuperscript{1,3}, H.X. Peng\textsuperscript{2}, X. Long\textsuperscript{2} and J.Y. Yao\textsuperscript{2}

\textsuperscript{1}Agricultural College, Guangxi University, Nanning 530005, China; \textsuperscript{2}Horticultural Research Institute, Guangxi Academy of Agricultural Sciences, Nanning 530007, China; \textsuperscript{3}Guangxi Crop Improvement and Biotechnology Lab, GXAAS, Nanning 530007, China

Wild banana germplasm is very valuable for breeding and crop improvement purposes. ISSR molecular markers technology was used to assess the genetic diversity and classification of about 30 wild and cultivated banana accessions collected in Guangxi. ISSR analysis showed that out of the 100 randomly chosen primers, 16 primers were polymorphic for the studied germplasm and obtained 168 stabilised amplified bands of which 84.5\% (142 bands) were polymorphic. A matrix was formed according to the presence (marked as 1) or not (marked as 0) of amplified bands for each primer and accession. Based on this, UPGMA clustering analysis was performed on the studied germplasm using the software NTSYS-2.10. Analysis showed that the studied accessions can be classified into four groups (coefficient level 0.64), including a group of \textit{Musa acuminata}, a group of \textit{Musa balbisiana}, a group of wild germplasm from Longzhou and cultivated accessions of the ABB group (Dajiao and Fenjiao). The wild germplasm collected in Guangxi showed a closer relationship to the ABB group than to \textit{M. acuminata} or \textit{M. balbisiana}, indicating most of them might be hybrids of \textit{M. acuminata} and \textit{M. balbisiana}. Analysis also showed that the comparability coefficient of wild germplasm in Guangxi ranged from 0.61 to 0.93 indicating a very high diversity. Wild germplasm from Pingxiang showed a very high comparability with \textit{M. balbisiana} indicating that Pingxiang germplasm might have the BB genome. However, the genotype of wild banana in Guangxi still needs to be characterised further by molecular as well as morphological tools.
Analysis of Genetic Diversity in Banana (*Musa* spp.) using Sequence-Related Amplified Polymorphism Markers

J.Y. Wei¹, D.B. Liu², S.X. Wei¹, Z.S. Xie¹, G.Y. Xu¹ and Y.Y. Chen¹

¹Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agriculture Sciences, Key Tropical Crops Germplasm Utilization, Ministry of Agriculture P.R. of China, Hainan, China; ²College of Horticulture and Forestry, Hainan University, Hainan, China

Most cultivated banana (*Musa* spp.) have evolved from interspecific hybrids of two wild diploid species, *Musa acuminata* Colla (AAₜ genome) and *Musa balbisiana* Colla (BBₜ genome). Molecular marker techniques can facilitate characterisation and differentiation of banana populations. In this paper, a novel molecular marker technique known as sequence-related amplified polymorphism (SRAP) was studied to determine diversity and relationships among 29 banana genotypes. The results showed that 25 out of 64 primer combinations were polymorphic and 324 polymorphic bands were obtained. Based on the appearance of the markers, the genetic relationships were analysed by the unweighted pair-group method of arithmetic average cluster analysis (UPGMA), and the genetic similarity coefficients were calculated. The results showed that most genetic relationships among cultivars were correlated to their region of origin. All 29 cultivars could be clustered into two major clusters closely corresponding with the genome composition of the genotypes (AA genome and hybrids between AA and BB genome). Some cultivars examined in this study did not cluster according to their hypothetical genetic homologies, which would suggest missampling or misidentification. All results indicated that the amplification of SRAP markers is useful for estimating genetic similarity among banana genotypes.
Study of Banana Germplasm by AFLP Based on the Technique of Capillary Electrophoresis

J.W. Zeng, X. Long, R. Xia, B.Z. Huang, Y.H. Huang, G.J. Yi

Fruit Tree Research Institute, Guangdong Academy of Agricultural Science, Guangdong 510640 China

The molecular marker tool AFLP is widely used in various research programmes, including genetic breeding, plant genome analysis, plant classification and evolution investigation. The results from AFLP can be detected either by denatured polyacrylamide gel electrophoresis or by capillary electrophoresis. Because of its higher separating efficiency, lower sample usage and better automatic operability, the latter technique is more used. In this study, 73 banana accessions were collected from the Chinese National Banana Germplasm Resource Centre in Guangzhou. After DNA isolation using the traditional CTAB method, PCR amplification was carried out with two AFLP primer pairs, EcoR ACC+Mse CAT and EcoR ACC+Mse CAG. Capillary electrophoresis was used to detect the amplified results. The analysis showed that 199 bands (81.2%) among the 245 bands amplified were polymorphic, indicating that there is wide genetic diversity in banana germplasm. The capillary electrophoresis results also showed that the 73 accessions could easily distinguished based on the characterised bands. According to the cluster analysis of AFLP fingerprinting, the 73 accessions could be divided into six groups (coefficient level 0.76), showing a high consistency with the genomic groupings of Simmonds, despite a few exceptions. ‘Fenjiao’ and ‘Dajiao’, though both classified as ABB in Simmonds grouping system, showed clear genetic difference, indicating that they do not have a close relationship and their classification may need to be revised. The fingerprinting also showed that ‘Jineiya’ and ‘Pingguo’, in spite of different names, are actually the same cultivar, which is consistent with the results of previous research. The good repeatability of the capillary electrophoresis revealed the high reliability of our results. AFLP technique based on capillary electrophoresis is an efficient and reliable method for determining genetic distinctness or relatedness among banana and plantain cultivars.
Studies on Intersectional Relationship between Eumusa and Rhodochlamys of the Genus *Musa* Using Morphotaxonomy and Microsatellite Markers

P. Durai\(^1\), S. Uma\(^1\), M.S. Saraswathi\(^1\), N. Jayabalan\(^2\) and M.M. Mustaffa\(^1\)

\(^1\)National Research Centre for Banana (NRCB-ICAR), Thogamalai Road, Thayanur (Post), Tiruchirapalli 620 102, Tamil Nadu, India; \(^2\)Department of Plant Sciences, Bharathidasan University, Tiruchirapalli 620 024, Tamil Nadu, India

The genus *Musa* of the family Musaceae has been classified into four sections, i.e. Eumusa, Rhodochlamys, Callimusa and Australimusa. Eumusa members have contributed a lot to the evolution of present day bananas. Rhodochlamys members, which are very close to the section Eumusa and more specifically to *Musa acuminata*, have a wide distribution in the Indian subcontinent. This paper deals with a detailed assessment of intersectional relationships using morpho- and molecular characterisation. A total of 24 accessions from Eumusa, Rhodochlamys and hybrids of Eumusa x Rhodochlamys were included in the study. All the test accessions were morphotaxonomically characterised and documented. A total of 21 primer pairs were used to assess the microsatellite polymorphism, of which 81% amplified product, resulting in discrete, reproducible amplicons. A total of 145 alleles were identified with a mean of 8.05 alleles per primer pair. An average PIC of 0.55 was observed in the present study. The developed dendrogram failed to project a clear-cut distinction between Eumusa and Rhodochlamys members, indicating a close genetic make-up as reported by Simmonds (1955) and Wong et al. (2002). Diversity analysis suggests that ‘Matti’, a Eumusa member, could be a parthenocarpic derivative of *M. acuminata* ssp. *burmannica*. Four *M. acuminata* wild forms, namely ‘Pagalaphad wild’, ‘Chengdawt’, ‘Lairawk’ and ‘Meghalaya wild’, which have been identified from North Eastern India during recent explorations, proved their uniqueness but exhibited higher genetic closeness to Rhodochlamys. *Musa laterita* and its progenies grouped in the same cluster in both morpho- and molecular characterisation, indicating their close genetic make-up.
Robustness of IRAP and RAPD Marker Systems in Studying the Intra-Group Diversity of *Musa* Cavendish (AAA) clones

M.S. Saraswathi, S. Uma, K. Prasanya Selvam, S. Ramaraj, P. Durai and M.M. Mustaffa

*National Research Centre for Banana (NRCB-ICAR), Thogamalai Road, Thayanur (Post), Tiruchirapalli 620 102, Tamil Nadu, India*

Banana belonging to the Cavendish subgroup (AAA) are commercially cultivated because of their high yield, short cropping duration and high economic returns. The field performance of exotic Cavendish cultivars, like ‘Grand Naine’, ‘Williams’ and ‘Dwarf Cavendish’, is proven superior due to their sturdy stature, high yield potential and better bunch quality. But morphological similarities between the four different types of Cavendish clones, namely dwarf, medium, tall and giant, makes their identification difficult under field conditions. Tissue-culture multiplication further aggravates the problem by way of induction of somaclonal variants. Hence, in the present study, ten IRAP primer combinations and 30 decamer RAPD primers were tested for studying the intra-group diversity. The average polymorphism exhibited by RAPD was 67.77% and by IRAP was 81.33%, indicating that there was substantial variation at the DNA level among the nineteen test accessions. However, IRAP markers grouped the tissue-culture variants of ‘Giant Cavendish’ in one and the same cluster with 95% similarities, while RAPD markers placed them apart in two different clusters. All dwarf Cavendish types, like ‘Williams’, ‘Dwarf Cavendish’, ‘Singapur’, ‘Jahaji’ and ‘Manjahaji’, clustered together in IRAP. Similarly, medium-tall types of Cavendish, namely ‘Harichal’, ‘Robusta’, ‘Shrimanti’ and ‘Pedda Pacha’, and the giant Cavendish types ‘Gandevi Selection’, ‘Grande Naine’ and ‘Madhukar’ grouped separately. Results suggest that IRAP is more robust than RAPD in studying the intra-group diversity of the Cavendish subgroup. Further, use of appropriate primer combinations might enable the development of fingerprints for use in genetic fidelity testing within Cavendish clones.
Studies on Genetic Resources of Seeded Banana in West Bengal

M.A. Hasan¹, R. Ray Chowdhury¹, M. Manna¹, K.K. Mandal¹, D. Majumder² and S. Jha³

¹Department of Fruits and Orchard Management, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India; ²Department of Agricultural Statistics, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India; ³Department of Agricultural Entomology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Thirty-four types of seeded bananas (Musa spp., BBB/BB) collected from different agroclimatic zones of West Bengal, India were characterised at the Horticultural Research Station, West Bengal, India during the years 2005-2008 by using 123 plant morphological characters with different multivariate techniques, such as proximity matrix, single-linkage hierarchical clustering of square Euclidean distance matrix, principal component analysis (PCA) and multidimensional scaling (MDS). The proximity matrix by both squared Euclidean and cophenetic correlation between types indicated high closeness/similarity between ‘Attiakala’ (BBB), ‘Bichkela 1’ (BBB), ‘Bichkela 2’ (BBB) and ‘Hill Banana’ (BBB). The highest proximity value of 20.62 showed maximum dissimilarity between ‘Kalyani Local-3’ (BBB), and ‘Maricha’ (BBB) and ‘Jhama Diara’ (BBB). A dendrogram using single-linkage clustering technique on squared Euclidean distance matrix and cophenetic correlation matrix showed 13 and 14 clusters, respectively. PCA was used by considering 13 factors on the basis of variance explained, i.e. more than 3%, and total explained variation was confined to 68%. Considering the dominant characters with positive loading under Factor 1, such as bract scar on rachis, pollen sacs colour, leaf corrugation, general fruit shape, fruits and compound tepal, the positively loaded types were ‘Bichkela 1’ (BBB), ‘Hill Banana’ (BBB), ‘Attiakala’ (BBB), ‘Bichkela 2’ (BBB), ‘Kalyani Local 1’ (BBB) and ‘Jhama Daira’ (BBB). Factor-1 thus explained 14.21% of total variance. In general, PCA results agreed with the results obtained by cluster analysis. PCA gave a better picture of the relationship between seeded banana types than cluster analysis and was useful in confirming group(s) obtained through cluster analysis. MDS group plots also clearly indicated the clustering
of homogeneous types. Among the 34 seeded banana types, 32 were assessed as parthenocarpic. The two non-parthenocarpic types identified were ‘Baruipur’ (BB) and ‘Seed Banana 15’ (BB).

**Generation of Mapping Populations for Segregation of ‘B’ Genome**

A. Rekha¹, K.V. Ravishankar² and D.S. Ambika

¹Division of Fruit crops, Indian Institute of Horticultural Research, Hessaraghatta, Bangalore 560 089, Karnataka, India; ²Division of Bio-technology, Indian Institute of Horticultural Research, Hessaraghatta, Bangalore 560 089, Karnataka, India

Two wild species, *Musa acuminata* (‘A’ genome) and *Musa balbisiana* (‘B’ genome), have contributed to the evolution of present-day banana cultivars. These cultivars have been classified into groups based on their morphological characters. There are however very few genetic studies available to support the morphological grouping, which may not always be very accurate. Modern molecular tools are available which will help in better characterisation, identification of hybridisation and analysis of evolutionary trends. Such studies require F1 populations. To develop a ‘B’ specific marker, both genomic groups were involved. Two wild species, *M. acuminata* ssp. *burmannicoides* ‘Calcutta-4’ (female parent) and *M. balbisiana* ‘Bee hee kela’ (male parent) collected from North-Eastern Hilly region of India, were crossed to produce segregating populations of the BB type. Seed set was very high (3552 seeds), and 317 seedlings germinated. About 150 seedlings were maintained for further evaluation and molecular mapping. Morphological parameters were observed regularly. The plant height was dwarf to medium, similar to ‘Calcutta-4’. The pseudostem was green with few black-brown blotches, expressing both characters, but less waxy, which is characteristic of the ‘A’ genome. For the 45 hybrids that flowered, most bunches were horizontal, showing the AB character. However, there was good segregation for male flower colour. The colour varied from cream (AA character) to pink-purple (BB character) with gradations of pink (42% of the observed progeny). It was observed that 40% of the F1 population studied did not produce any pollen and were totally sterile, unlike the parent accessions. These F1 populations could serve as useful material for studying segregation of different morphological characters and molecular mapping.
Genotyping of Dessert Banana Cultivar ‘Kolikuttu’ (AAB, Silk)

W.L.G. Samarasinghe¹, H.W.L. Pushpakumari², J.L.P. De Silva² and S.G.J.N. Senanayake²

¹Plant Genetic Resources Centre, Gannoruwa, Peradeniya, Sri Lanka; ²Faculty of Agriculture, University of Ruhuna, Sri Lanka

Banana (Musa spp.) is the most important and widely consumed fruit in Sri Lanka. ‘Kolikuttu’ (AAB) is one of the most highly priced dessert cultivars in the local market. Being a traditional cultivar, ‘Kolikuttu’ shows some apparent morphological variation in the field but its genetic variation that could be used for genetic improvement is not known. Twenty-three (23) accessions of ‘Kolikuttu’, collected based on morphological variations and Fusarium wilt tolerance and from tissue-culture plants from different mother plants, were genotyped using SSRs. DNA of these accessions was subjected to PCR using six SSR primers, namely MaSSR 24, MaSSR 20, MaSSR 18, Mb1- 69, Mb1- 113 and Mb1- 134. PCR products were separated by PAGE and the products were detected by Silver Staining. Bands were visually scored and statistically analysed. A total of 26 alleles were detected of which 73.08% were polymorphic. A genetic distance matrix and a dendrogram were derived. The dendrogram showed five different ‘Kolikuttu’ types. Despite higher morphological variation seen among accessions, lower genetic variation was shown with respect to the analysed SSR loci. However, some morphotypes for fruit characteristics were identified as different DNA types. Two genotypes were identified with Fusarium wilt tolerance. Tissue-culture plants derived from different mother plants were genetically dissimilar.

Genetic Structure of Musa acuminata (AA) Populations in Sri Lanka Revealed by SSR Markers

W.L.G. Samarasinghe and S.L.D. Jayaweera

Plant Genetic Resources Centre, Gannoruwa, Peradeniya, Sri Lanka

Musa acuminata is a wild ancestor of most cultivated banana and plantain. The species has great value for crop improvement programmes because of its rich
genetic diversity and the presence of viable seeds. In Sri Lanka, *Musa acuminata* occurs in areas from 179 to 1500 masl in the forests in ‘Knuckles Matale’ (population 1), ‘Knuckles Kandy’ (population 2) and ‘Saptha Kanya’ and Yatiyantota (population 3). Information on genetic diversity and genetic structure is of great value for in-situ conservation, germplasm collection missions, set-up of core collections, evaluation of germplasm and banana breeding activities. SSR analysis was carried out using five primer pairs for samples representing the three populations. SSR loci visualised on silver-stained 8-10% denaturing PAGE gels were analysed using GenAlEx 6 and SPSS 10. Percentages of polymorphic loci for population 1, 2 and 3 were 20%, 40% and 60%, respectively. AMOVA analysis showed 19% variance among and 81% within populations. Nei’s Genetic distance increased along with increased geographic distance. The mean $H_O$ (0.2727) and mean $H_E$ (0.1943) indicates that outbreeding occurs in *M. acuminata* populations. Bats and bees are the pollinators. However, the presence of few fruits in bunches of *M. acuminata* reflects that there is a barrier for cross pollination which limits the seed formation and thereby seed propagation. Lower level of allele migration rate ($N_m = 0.784$) and higher $F_{ST}$ (0.242) was observed between distantly located populations 1 and 3 (aerial distance about 69 km) in contrast with the closely located (aerial distance about 24 km) populations 1 and 2 ($N_m = 1.640$ and $F_{ST} = 0.132$).

**Post-Harvest Characterisation of Three Banana Cultivars from the CARBAP Musa Germplasm Collection in Cameroon**

G. Ngoh Newilah¹, C. Dhuique-Mayer², K. Tomekpe¹, E. Fokou³ and F.X. Etoa³

¹Centre Africain de Recherches sur Bananiers et Plantains de Njombé, 832 Douala, Cameroon; ²CIRAD – Dept PERSYST/UMR 95, Integrated Food Processing Research Unit, 73 av. J.F Breton 34398 Montpellier CEDEX 5, France; ³Department of Biochemistry, University of Yaoundé I, 812 Yaoundé, Cameroon

Banana and plantain are an essential component of the diet in Cameroon, where they are widely consumed as fresh fruit or after being processed. A better understanding of their composition may help processors to improve processing techniques and to obtain good-quality products. It may also be of great
importance to breeders. Three Musa cultivars [a plantain ‘French Sombre’ (AAB), a cooking banana ‘Pelipita’ (ABB) and a Cavendish dessert banana ‘Grande Naine’ (AAA)] were characterised and evaluated for some post-harvest parameters. From early fruit development to optimal physiological maturity, pulps were collected from the 2nd and 3rd hands of at least three ‘on-plant’ bunches every 14 or 21 days depending on the Musa subgroups. During ‘off-plant’ ripening, fruits were also sampled at specific maturity stages and analysed for some physicochemical characteristics (pulp to peel ratio, total soluble extract, total titratable acidity, pulp and peel dry matter content, pulp firmness, pH, pulp and peel color) and micronutrient content [especially total carotenoid content using spectrophotometric (\(\lambda = 450\) nm) techniques, and lutein, \(\alpha\) - and \(\beta\)-carotene using chromatographic (HPLC) techniques]. From shooting to harvest, the physicochemical parameters of the analysed pulps varied significantly \((P < 0.05)\). During post-harvest maturation of these Musa pulps, their nutritional content also showed different trends. The concentrations of the evaluated parameters and trends differed significantly according to Musa subgroups during fruit filling and ripening. The high carotenoid content of plantain and cooking banana cultivars confirmed the interest of these staple crops as potential sources of provitamin A whose anti-oxidant properties are essential in human health.

**Determination of Nutritional Composition of Four Banana Cultivars Available in the Market of Up-Country Intermediate Zone of Sri Lanka**

M.A.L.N. Mallawaarachchi

*Regional Agriculture Research and Development Centre, Bandarawela, Sri Lanka*

Banana (Musa spp.) is a widely grown and consumed semi-perennial fruit crop in Sri Lanka. There are more than twenty cultivars grown throughout the country as dessert fruit and as cooking banana. ‘Ambul’ (AAB, Mysore) and ‘Seeeni Kesel’ (ABB, Pisang Awak) are the most popular dessert varieties, while ‘Ash Plantain’ (ABB) is the most preferred cooking banana in the Up-Country Intermediate Zone of Sri Lanka (altitude 1000-1500 m, annual rainfall 1500-2000 mm). These cultivars are in high in demand due to their low price and
high availability. Though they are widely consumed, little is known about their nutritional value. Therefore, a study was conducted at the consumable stage (i.e. mature stage for ‘Ash Plantain’ and ripening stage for the others) for ascorbic acid, potassium, moisture, total soluble solids (TSS) contents, pH and flesh-to-peel ratio, and compared with the highly priced cultivar ‘Anamalu’ (AAA, Cavendish). Samples were collected from a local market which receives fruits only from a unique environmental condition (Up-Country Intermediate Zone). Ascorbic acid content ranged from 8.355 mg/100g in ‘Anamalu’ to 3.129 mg/100g in ‘Seeni Kesel’ which also contains highest flesh-to-peel ratio (4.378), TSS (27.380) and potassium (196.875 mg/100g) contents. The highest moisture content was recorded in ‘Anamalu’ (76.44%). The high potassium level of ‘Seeni Kesel’ may be an advantage over the cultivar ‘Anamalu’ for therapeutic use. Iron deficiency is a common health problem in the area, therefore evaluation of ascorbic acid content in these banana cultivars is vital because it induces iron absorption of the diet and acts as an anti-oxidant.

**Fruit Quality of Some Banana Cultivars Grown in Tunisian Coastal Oases of Gabes**

A. Ferchichi\(^1\), M. Ben Salah\(^2\) and M. Jeridi\(^1\)

\(^1\)Institut des Régions Arides, 4119, Médenine, Tunisia; \(^2\)Institut des Régions Arides, Nahal 6051 Gabes, Tunisia

Banana (Musa spp.) is cultivated in the coastal oases of Tunisia, characterised by a Mediterranean climate with moderate temperature. Six locally grown cultivars have been described: ‘Arbi1’ (AAA), ‘Arbi2’ (AAA), ‘Guebsi Jwaid’ (ABB), ‘Guebsi Khchin’ (ABB), ‘Spani’ (ABB) and ‘Lobnani’ (ABB). Those cultivars were described morphologically. Fruits of plants of these cultivars grown under the same conditions in oases were harvested at the same stage of development. Fruit chemical composition of important components was determined. Brix degree, pH and total acidity were determined, respectively by manual refractometer, pH meter Thermorion 410A and titrating method (KOH 1N). Fruit water content, reducing sugars and Vitamin C were determined by HPLC. Total polyphenols and minerals composition (Mg, K, Na, Mn, Fe, Zn, Mg, Cu) were analysed by atomic absorption (SHIMADZU AA 6800 material). P and polyphenols were determined by spectrophotometry. The fruit chemical
composition showed variability between cultivars, especially in reducing sugar and TSS of the fruit. ‘Spani’ and ‘Arbi2’ are the two sweetest cultivars with rates of 5.9%. ‘Gabsi Jwaid’ was the least sweet with 2.5%. A wealth of K has been detected in the majority of cultivars with a max of 1.5% in ‘Arbi2’ and a min of 0.9% of dry weight in ‘Spani’. The banana cultivars also had relatively low levels of ascorbic acid in comparison with orange and other tropical fruits. The highest content was recorded in ‘Spani’ with 8.79 mg/100g, the lowest for ‘Lobnani’ 0.5 mg/100g of dry weight.

Expression of MaMADS2 and Its Interactions with Ethylene Suggest that It Acts Upstream to Ethylene Production

T. Elitzur1,2, E.E. Goldschmidt2, J. Giovannoni3, J. Vrebalov3 and H. Friedman1

1Dept. of Postharvest Science of Fresh Produce, ARO, The Volcani Center, Bet Dagan 50250, Israel; 2Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture, The Kennedy-Leigh Centre for Horticultural Research, Faculty of Agriculture, Food and Environmental Quality Sciences, Hebrew University of Jerusalem, Rehovot, Israel; 3U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS) Robert W. Holley Center and Boyce Thompson Institute for Plant Research, Cornell University, Ithaca, NY 14853, USA

Several members of the MADS-box gene family (MaMADS1-6) were cloned from banana cultivar ‘Grand Nain’ (AAA, Cavendish). Based on its expression patterns and interactions with ethylene, only MaMADS2 is suggested to be a candidate master regulator of ripening in banana. Expression of MaMADS2 is higher in the pulp than in the peel, and the increase in expression of MaMADS2, together with MaMADS3, 4 and 5, preceded ethylene production, but co-incided with the CO2 respiration peak in the pulp. On the other hand, in the peel, the expression of the genes MaMADS2 together with MaMADS1, 3 and 4 coincided with increased ethylene production. Ethylene applied early after harvest and 1-MCP applied at the onset of increase in ethylene production did not affect the levels of MaMADS2, examined immediately after treatment, although these treatments affected the expression levels of the other MaMADS-box genes either in peel or in pulp. On the other hand, application of high levels of CO2 immediately after harvest reduced the levels of MaMADS2 as well as a number of the other MaMADS-box genes. These results suggest that MaMADS2
expression is not dependent on ethylene, but possibly on mitochondrial function. Although the gene showed only low homology to tomato RIN, we suggest that MaMADS2 may function similarly to RIN, acting first in the pulp, possibly with other MaMADS-box proteins to activate the ripening program. Transgenic banana plants down-regulated in MaMADS2 and MaMADS1 were created and should bear fruit shortly.

**Compositional Characteristics and Nutritional Evaluation of the Male Flower Bud from Two Chinese Banana Cultivars, ‘Baxijiao’ (AAA) and ‘Paradisical’ (AAB)**

Z.W. Sheng, W.H. Ma, Z.Q. Jin, J.H. Gao, J.Y. Li and L. Han

*Haikou Experimental Station, Chinese Academy of Tropical Agricultural Sciences, 570101 Haikou, Hainan, P. R. China*

The male flower buds of two banana cultivars, ‘Baxijiao’ (AAA) and ‘Paradisical’ (AAB), cultivated in Hainan Province of China, were analysed to determine their composition with regard to mineral elements, vitamin E profile, total flavonoids and saponin, free fatty acids and amino acid content. Investigations showed that banana flower contained 89.42-90.58 g/100g moisture, 1.62-2.07 g/100g protein, 0.4-0.6 g/100g fat, 1.19-1.24 g/100g ash, 4.96-5.74 g/100g total dietary fiber. Major amino acid components were glycine, aspartic acid, leucine and alanine acid. Among the essential amino acids assayed, lysine with a chemical score of 64% appeared to be the most limiting when compared with the essential amino acid pattern of egg protein. Mineral composition showed that sufficient amounts of Mg, Fe and Cu were present to meet the macronutrient and micronutrient demand in human diets. The flowers contained a high composition of unsaturated fatty acids (65-66%), mainly linoleic acid, and of saturated fatty acids, mainly palmitic acid. The contents of vitamin E, total saponin and flavonoids were found to be 0.87-1.07 mg/100g, 0.12 mg/100g and 5.27-5.90 mg/100g, respectively. This study was conducted to create nutritional data for banana flower in order to popularise its consumption and utilisation in China. The banana flower bud is already a popular delicacy in many Asian countries, like Sri Lanka, Indonesia and Thailand.
Roles of Soluble Sugars on Degreening of Banana Fruit

L.Y. Xu¹, X.T. Yang¹, Z.Q. Zhang¹, R.Q. Fang² and X.Q. Pang²

¹College of Horticulture, Key Lab of Postharvest Science of Guangdong Province, South China Agricultural University, Guangzhou, China; ²College of Life Science, South China Agricultural University, Guangzhou, China

Banana fruits (AAA genome) fail to develop a yellow peel and stay green when ripening at warm temperatures above 24°C. These “green-ripe” fruits are perceived to be of poor quality and consequently fetch a lower price. In a previous study, we demonstrated that chlorophyll (Chl) degradation is strongly suppressed when the fruit ripens at 30°C compared with ripening at 20°C. To study the mechanisms by which temperature interferes with Chl degradation is of both biological and practical significance. Faster accumulation of high-level fructose and glucose in the peels at 30°C prompted us to investigate the roles of soluble sugars on Chl degradation by in-vitro incubation of detached banana peel pieces. After incubation without sugar for 8 days, detached banana peel pieces turned yellow at 30°C as well as at 20°C. Low concentrations of 20 mM fructose or glucose did not suppress piece degreening at either temperature. However, when sugar concentration increased to 50, 100 and 250 mM, the pieces stayed green after 8 days both at 20°C or 30°C and retained around 30 μg/gfw of Chls, 2-fold higher than retention in pieces that were incubated without sugars. Manitol at 100 and 250 mM did not inhibit piece degreening, which rules out the possibility that the inhibitive effect of sugars on degreening is due to their osmotic effect. A similar inhibitive effect on banana piece degreening as fructose or glucose was observed for mannose, but not for a glucose analogue, 3-O-methyl-glucose, which was proven to be not phosphorylated by hexokinase. The results indicate that sugars, and not high temperatures, interfere with chlorophyll degradation in detached banana peel in vitro, and that increased sugar level in peels at high temperatures may be a direct factor leading to Chl catabolism repression in vivo. Sugar signaling mediated by hexokinase might function in the degreening suppression by sugars.
Pre-Breeding Bananas: Identifying Genotypes with Enhanced Functional Food Attributes

E. Amorim¹, K. Cohen², V. Amorim¹, N. Paes², H. Souza², J. Santos-Serejo¹ and S. Silva¹

¹Embrapa Cassava and Tropical Fruits, Cruz das Almas, Brazil; ²Embrapa Genetic Resources and Biotechnology, Brasilia, Brazil

The objective of this work was to determine the concentration of polyphenols, flavonols, vitamin C and carotenoids in 61 banana accessions from the Musa germplasm collection at Embrapa Cassava and Tropical Fruits, Brazil. Significant differences were detected between accessions for all characteristics analysed by ANOVA. The average polyphenols content was 38.06 mg/100g, ranging from 12.51 mg/100g in ‘Torp’ (AAA) to 257.80 mg/100g in ‘Teparod’ (ABBB). The average flavonols content was 2.10 mg/100g, ranging from 0.85 mg/100g in ‘Maravilha’ (AAAB) to 6.63 mg/100g in ‘Teparod’. The average vitamin C content was 21.61 mg/100g, ranging from 8.60 mg/100g in the tetraploid ‘Bucaneiro’ (AAAA) to 76.82 mg/100g in ‘Teparod’. The average total carotenoids content was 4.20 g/g, ranging of 0.97 g/g in ‘Tropical’ (AAAB) to 19.24 g/g in ‘Saney’ (AAB). ‘Modok Gier’ (AA) and ‘NBA-14’ (AA) had 7- and 5-fold higher carotenoids content than the representatives from the Cavendish group (‘Nanica’, ‘Williams’ and ‘Lacatan’, AAA). The genetic variation in the concentrations of these functional components can be exploited to obtain new cultivars with higher levels of these functional components through crossings and selection. Cultivars with higher contents of vitamins, vitamin precursors and flavinols have the potential of neutralising free radicals and preventing diseases, including some types of cancer.
Sequence Comparison of cDNAs and Expression Analysis of Phenylalanine Ammonia-Lyase from Different Banana Cultivars after Infection with *Mycosphaerella fijiensis*

J. Correa\(^1\), A. Rodríguez\(^2\), E. Rodríguez-Arango\(^3\), Z.I. Monsalve\(^4\) and R. Arango\(^5\)

\(^1\)Plant Biotechnology Unit UNALMED-CIB, Corporación para Investigaciones Biológicas, Medellín, Colombia; \(^1\)Laboratory of Plant Molecular Biology, Instituto de Biología, Universidad de Antioquia, Medellín, Colombia; \(^2\)Plant Biotechnology Unit UNALMED-CIB, Corporación para Investigaciones Biológicas, Medellín, Colombia; \(^3\)Plant Biotechnology Unit UNALMED-CIB, Corporación para Investigaciones Biológicas, Medellín, Colombia; \(^4\)Laboratory of Plant Molecular Biology, Instituto de Biología, Universidad de Antioquia, Medellín, Colombia; \(^5\)Plant Biotechnology Unit UNALMED-CIB, Corporación para Investigaciones Biológicas, Medellín, Colombia; \(^5\)Facultad de Biociencias, Universidad Nacional de Colombia sede Medellín, Colombia

Black leaf streak, caused by the fungus *Mycosphaerella fijiensis*, is the most important disease affecting the commercial production of banana and plantain in the world. Damage by this fungus has resulted in substantial economic losses and a significant increase in annual production costs. Phenylalanine-ammonia-lyase (PAL) is an enzyme found in a large group of plants, which catalyses the first reaction in the metabolic pathway of phenylpropanoid compounds. Phenylphenalenons, a type of phenylpropanoid metabolites, have been found in banana and have been suggested to be involved in resistance against pathogens. Thus, PAL might be an important enzyme in the plant-pathogen interaction in banana. In this work, complete PAL cDNAs were isolated and sequenced from ‘Calcutta 4’ (*Musa acuminata* ssp. *burmannicoides*) and ‘Grain Nain’ (AAA, Cavendish subgroup), whereas partial cDNAs were obtained from ‘Yangambi Km5’ (AAA, Ibota subgroup) and ‘Williams’ (AAA, Cavendish subgroup). Complete PAL cDNA from ‘Calcutta 4’ has a size of 2.8 kb and encodes 712 amino acids. All the obtained sequences were compared, translated into protein and their secondary structure modeled. Several structural differences between cultivars were found. Additionally, expression studies using semi-quantitative RT-PCR showed marked differences in the basal expression of PAL depending on the cultivar. Furthermore, black leaf streak-resistant ‘Calcutta 4’ showed a high peak of PAL expression present in early hours of infection. In contrast, there was a constant level of expression in the susceptible cultivar ‘Williams’
during these early stages of infection. This work contributes to better understanding the *M. fijiensis*-banana interaction, at molecular level. In the long term, biotechnological strategies could be designed to modulate the amount and type of phenylphenalenons, thus enhancing resistance to black leaf streak in commercial susceptible cultivars.

**Gene Expression Analysis in Leaves of ‘Bee Hee Kela’, a Drought Tolerant Banana Genotype**

K.V. Ravishankar, A. Rekha, R.H. Laxman, G. Savitha and V. Swarupa

*Indian Institute of Horticulture Research, Hesaraghatta Lake Post, Bangalore 560 089, India*

Drought is the most limiting environmental factor for plant growth and crop productivity. Understanding the mechanism behind drought tolerance and efficient water use by the plant is necessary to cope with drought stress. In this study, contrasting genotypes for drought tolerance in banana were identified based on physiological characteristics, such as gas exchange characteristics, leaf water retention capacity (LWRTC), root characteristics. Among the contrasting genotypes of a *Musa balbisiana* collection from North East India, ‘Bee Hee Kela’ (BB) was shown to be more drought tolerant. Suppression subtractive hybridisation was carried out to identify differentially expressed genes in leaves of ‘Bee Hee Kela’ during water stress. Nearly 200 clones were sequenced and 50 non-redundant sequences were identified. They were characterised based on homology search in National Center for Biotechnology Information (NCBI) database. BLASTx search results showed higher similarity with lipoxygenase, rubisco activase, glycine dehydrogenase, catalase and ethylene responsive factor (ERF). These genes might be involved in many cellular functions, such as cell membrane integrity, signal transduction and metabolism in response to dehydration.
Studies on Time-Course Expression of Defense Gene in Banana against Pratylenchus coffeae for Creation of Subtractive cDNA Library

S. Backiyarani, S. Uma, P. Sundararaju, M. Mayilvaganan, M.S. Saraswathi and S. Jeeva

National Research Centre for Banana (NRCB-ICAR), Thogamalai Road, Thayanur (Post), Tiruchirapalli 620 102, Tamil Nadu, India

Identification of genes that confer nematode resistance is an important factor in banana improvement programmes. These can either be used as molecular markers for marker-assisted selection or directly by guiding the design of transgenic plants with high resistance. Cultivars with differential reaction to nematode infection and knowledge on time-course expression of defense genes after nematode infection are a prerequisite for the isolation of resistance genes in banana through functional genomics. Based on pot-culture screening and biochemical studies, cultivars ‘Karthombiumtham’ (ABB) and ‘Nendran’ (AAB) were identified as resistant and susceptible, respectively, to the root-lesion nematode Pratylenchus coffeae. The semi-quantitative RT-PCR analysis revealed that mRNA levels of the chalcone synthase gene, the first enzyme in the pathway for flavonoid biosynthesis, was constitutively higher in roots of the resistant ‘Karthombiumtham’ than in the susceptible ‘Nendran’. The transcript level of this defense gene was found to be higher up to 6 days after nematode inoculation (DAI) and started declining from 7 DAI onwards in both resistant and susceptible root samples. From these preliminary studies, it is inferred that a subtractive cDNA library should be created from the collected root samples, within 6 DAI of P. coffeae. A cDNA library is being created through Suppression Subtractive Hybridisation (SSH) for isolating the genes that are differentially activated during the nematode host interactions.
Isolation of a Cyclin D2;1-type Gene Homologue from East African Highland Banana (*Musa*, AAA)

D. Talengera¹, A. Kiggundu¹, W.K. Tushemereirwe¹, D. Inze² and K. Kunert³

¹National Banana Research Programme, National Agricultural Research Laboratories, Kawanda, PO Box 7065, Kampala, Uganda; ²Department Plant Systems Biology (VIB), Technologiepark 927, 9052 Ghent, Belgium; ³Forestry and Agricultural Biotechnology Institute, University of Pretoria, 0002 Pretoria, South Africa

Knowledge of the level of homology of *Musa* genes to that of characterised plant genomes provides a basis for translating genomic technologies into molecular breeding of *Musa*. In this study, a full cDNA of cyclin D2;1 gene, one of the genes modulating the progression of the cell cycle, from an East African highland banana cultivar ‘Nakasabira’ (AAA), a dessert cultivar ‘Sukalindiizi’ (AAB) and one of their progenitors, ‘Calcutta 4’ (*Musa acuminata* spp. *burmannicoides*) was isolated through comparative sequence analysis and combined RACE and genome walking techniques. The 344-amino-acid sequence was common to the three *Musa* genotypes, had typical cyclin N- and C-terminal domains, an LLCAE retinoblastoma-related protein-binding motif at the N-terminus and a characteristic IWKVHAY signature motif. Amino-acid sequence comparison revealed a 50%, 54%, 55% and 58% identity with *Arabidopsis*, rice, maize and wheat cyclin D2;1, respectively. Polymerase chain reaction with primers designed from the flanking regions of the full cDNA nucleotide sequence amplified a full genomic cyclin from a wide range of banana genotypes but with no signal in tobacco and maize, confirming the isolated sequence as a *Musa* cyclin D2;1. This sequence may be an additional resource to the ongoing *Musa* genomics research. Cyclins have received considerable scientific scrutiny because of their role in modulating the cell cycle that determines plant growth and development. Based on results from model plants, we discuss the potential of upregulating cyclin genes to increase cell division and shorten the maturation process of banana as a strategy for increasing productivity.
Seed as an Alternative Source of DNA for Molecular Research of Inaccessible Wild Musa species

S. Uma, M.S. Saraswathi and D. Anto

National Research Centre for Banana (NRCB-ICAR), Thogamalai Road, Thayanur (Post), Tiruchirapalli 620 102, Tamil Nadu, India

Most wild species and wild relatives of banana (Musa spp.) are found in forests and are thus relatively inaccessible. Their inability to establish in places different from their natural habitats further complicates the use of such wild species for routine molecular research. The present study was therefore undertaken to evaluate seeds as an alternative source of DNA for conservation in comparison with the cigar leaf. Usefulness of seed as a major genetic tool for conservation of seeded wild banana germplasm, standardisation of the stage of seed maturity for DNA extraction and standardisation of the seed DNA extraction protocol were studied. The accessibility of seed DNA as an alternative strategy for leaf DNA was confirmed using molecular markers (RAPD and IRAP). Among different methods of DNA extraction, a modified protocol with CTAB was found to be the best. Partially mature seeds with 70-80% maturity were found to be the best stage for DNA extraction compared with 40-50% and 100% maturity. Removal of the seed coat improved the quality of DNA extracted. Uniformity of seed and leaf DNA was confirmed by using 80 random primers and 10 inter retro transposon (IRAP) primers. The paper tries to analyse the extent of deviation between seed and leaf DNA and possible reasons in view of the breeding behaviour of wild species.

Role of In-Vitro Selection of Multiple Bud Clumps in the Screening of Fusarium Wilt Resistant Somaclones of Bananas in Taiwan

S.W. Lee, S.Y. Lee and M.J. Huang

Taiwan Banana Research Institute, Pingtung, Taiwan

Multiple bud clumps (MBCs) were propagated in MS medium containing Thidiazuron and Paclobutrazol. The whitish compact clusters of tiny meristems
were used for the induction of tolerance to fusarial toxin in tissue culture. Crude culture filtrate of *Fusarium oxysporum* f. sp. *cubense* race 4 (Foc race 4) (10 to 15%) or commercially available fusaric acid (0.1 to 0.25 mM) was added to the selection medium. We studied the survival rate of MBCs in selection medium containing various concentrations of fungal toxin after two to five successive cycles of subculture. Plantlets regenerated from the tolerant MBCs were transplanted in potting mix inoculated with Foc race 4 spores for the screening of disease resistance in the nethouse. Plantlets without Fusarium wilt symptoms were planted in a Foc race 4 infested orchard for the screening for disease resistance. In the preliminary field trials, among the 1922 clones obtained from different selection cycles and different cultivars, 74 clones did not show disease symptoms 6 to 8 months after planting in a heavily infested orchard designated for Fusarium wilt screening. Tissue-culture plantlets were propagated from suckers of these selected clones for further confirmation of stability in disease resistance, horticultural traits and post-harvest fruit quality. The screening programme of the Taiwan Banana Research Institute has released four commercial Fusarium wilt resistant Cavendish cultivars through the selection and improvement of somaclonal variants from tissue-culture plants. Through the in-vitro selection of MBCs tolerant to the fungal toxin, we can screen a very large number of compact meristems from target cultivars with reduced space and manpower. We envision that the in-vitro selection of tolerant MBCs, in combination with the nethouse and field selection scheme, will accelerate the progress of our screening programme for Fusarium wilt resistant cultivars.

**In-Vitro Mutagenesis for Banana Improvement**

S. Mohan Jain

*Department of Applied Biology, University of Helsinki, PL 27, Latokartanonkaari 5, Helsinki, Finland*

Banana and plantain (*Musa* spp.) are the fourth most important food crop in the world. They are grown in more than 100 countries with an annual production of around 90 million metric tonnes, they grow in a range of environments and produce fruit year-round. Banana fruit production is threatened by several major diseases and pests, such as black leaf streak, Fusarium wilt and the
burrowing nematode. Breeding of edible banana is hampered by high sterility, triploidy and lack of seed set. Few diploid banana clones produce viable pollen, however, the major problem with *Musa* germplasm enhancement is its low reproductive fertility and slow propagation rate. Biotechnology and gene technology, together with mutagenesis and conventional propagation methods can assist in overcoming these problems in developing new banana cultivars. In-vitro propagation of banana is successful and is routinely used for clonal propagation by commercial companies. On average, annually several millions in-vitro plants are produced. Somatic embryogenesis of banana has progressed, but is however not yet commercially exploited due to highly genotypic dependence. Somatic embryogenic cell suspension is highly suitable for mutation induction and genetic transformation. The progress on banana protoplasts and doubled haploids is very slow. Protoplasts would be an ideal system for developing asymmetric and symmetric somatic hybrids, especially for partial genome transfer. Nuclear techniques have been used for mutation induction in 154 plant species, both seed and vegetative propagated crops (http://www.-mvd.iaea.org), for their genetic improvement. In our Coordinated Research Project on banana at IAEA, Vienna, Austria, several mutants were isolated for several traits, namely reduced height, tolerance to Fusarium wilt, early flowering, large fruit size and black leaf streak tolerance (under multi-location field trials). This review presentation will discuss prospects of application of molecular tools for marker-assisted selection and breeding, TILLING together with an overview of banana biotechnology and mutagenesis.

**Synchronisation of Somatic Embryos by Liquid Medium-Based Protocol for ‘Rasthali’ (*Musa*, AAB)**

S. Uma, A. Akbar, M. S. Saraswathi, and K. Udhayaanjali

*National Research Centre for Banana (NRCB-ICAR), Thogamalai Road, Thayanur (Post), Tiruchirapalli 620 102, Tamil Nadu, India*

Difficulties in the application of somatic embryogenesis for plant propagation and genetic improvement of insufficiently characterised *Musa* genotypes may arise due to lack of complete knowledge about the factors controlling them. Uniformity in embryo production through synchronisation in development is very important in the field of propagation and genetic engineering. The present
study was undertaken to improve the conversion percentage of banana embryogenic cell suspensions (ECS) through a modified regeneration process. An ECS of ‘Rasthali’ (AAB) was initiated using immature male flower buds as explant (INIBAP technical guidelines). The suspension was maintained in complete darkness at 80 rpm. After 3 months, the suspension constituted mainly of aggregates (60 to 80% of the suspension) which were used as base material for regeneration and genetic transformation. Packed cells (0.5 ml) were plated on regeneration medium (Schenk and Hildebrandt medium), and the cultures were incubated at 27ºC under complete darkness without subculturing. After 60 days, one set of embryos was transferred to standard solid germination medium, while another set was transferred to regeneration medium with an intermittent treatment of liquid regeneration medium for 20 days. Better efficacy of liquid regeneration medium resulted in enhanced conversion capacity of embryos into plantlets (89% as against 70.5% in the standard method). Plants regenerated in both methods, but synchronisation and better regeneration was observed when they were regenerated from uniformly mature and viable embryos by the use of synchronisation enhancement treatment.

Histological Analysis of Somatic Embryogenesis in the Banana Cultivar ‘Mas’ (AA)

Y.R. Wei1,2, G.J. Yi1, C.H. Hu1, B.Z. Huang1, C.Y. Li1 and X.L. Huang2

1Fruit Tree Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou, 510640, China; 2The Key Laboratory of Gene Engineering of Ministry of Education, School of Life Sciences, Zhongshan (Sun Yat-sen) University, Guangzhou, 510275, China

Histological analysis was performed to elucidate the origin and the developmental process of somatic embryos derived from immature male flowers of the banana cultivar ‘Mas’ (AA). A total of 1725 individual male flowers from 115 male inflorescences were used to induce the embryogenic callus. The investigation revealed that a yellow and friable embryogenic callus could be obtained from individual flowers after 5 months on callus induction medium, and 7.5% embryogenic callus could be induced from these individual flowers. The embryogenic callus was used to initiate a suspension culture, and
a homogenous embryogenic cell suspension (ECS) was established after 3 months culture. ECS cultured for 6 months was transferred to semi-solid medium to induce somatic embryos. After 5 days of culture, bicellular and multicellular pro-embryos were observed. An irregular protoderm was differentiated into multicellular pro-embryos after 15 days culture. After another 5 days culture, pro-embryos developed into early globular embryos, which successfully went through the stages of oblong embryo, pear-shaped embryo and cotyledon-shaped embryo. Mature embryos were formed after 3 months culture, and an epidermis, shoot apex meristem, root apex meristem and central vascular zone could be distinguished. Mature embryos germinated 10 days after transfer to germination medium. Thirty days later, healthy plantlets were obtained from the germinated embryos.

**Genetic Transformation of GUS Gene into the Banana Cultivar ‘Pisang Mas’ (AA) by Agrobacterium-Mediated Transformation**

C.H. Hu, Y.R. Wei and G.J. Yi

*Fruit Tree Research Institute, Guangdong Academy of Agricultural Science, Guangzhou 510640, China*

In order to develop a highly efficient Agrobacterium-mediated genetic transformation protocol for banana, we explored the effect of different concentrations of two antibiotics and developed a new protocol of liquid-medium selection system for Agrobacterium-mediated transformation. The results showed that hygromycin is more sensitive than kanamycin, with the suitable concentration of kanamycin on liquid-medium cultivation of embryogenic cell suspensions (ECS) being 50 mg/L, compared to 15 mg/L for hygromycin. Male-flower-derived ECS of ‘Pisang Mas’ (AA), a major banana cultivar in Southeast Asia, were cocultivated with Agrobacterium strain EHA105 harbouring a binary vector pCAMBIA1301 carrying nptII and gusA gene in M2 liquid medium supplemented with 100 μM ACS. The infected ECS were cocultivated first for 1 day at 60 rpm and then for 4 days at 110 rpm on a rotary shaker. After 5 days of cocultivation in liquid medium, the infected ECS were cultivated in M2 selective liquid medium containing suitable
concentration of cefotaxime and hygromycin and subcultured every 10 days. Fine transgenic cell suspensions were obtained within 50 days of selection and confirmed by histochemical GUS assay. Regeneration of transformants after liquid selection cultivation was carried out according to common method, except for the absence of antibiotic in the medium. Compared to semisolid cocultivation and semisolid selection transformation in other banana species, it was found that liquid cocultivation and selection was more efficient for the transformation of ECS of banana.

Production of Suitable Target Tissues for Banana Transformation Studies

M. Maziah¹, S. Sreeramanan¹² and M. Sariah³

¹Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia; ²School of Biological Sciences, Universiti Sains Malaysia, Minden Heights, 11800, Georgetown, Penang, Malaysia; ³Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia. 43400, Serdang, Selangor DarulEhsan, Malaysia

Multiple bud clumps (MBCs) are whitish compact clusters of proliferating shoot meristems. A single bud from MBCs can be good target material for genetic transformation studies and a potential source for embryogenic cell suspension cultures in banana. Single buds are the materials of choice to be used as target tissues for Agrobacterium- and particle bombardment mediated-transformation in banana cultivar ‘Rastali’ (AAB) because they are easily propagated in vitro, potentially regenerable tissue and could reduce the possibility of chimeric plants since each bud will regenerate into a single shoot. In this study, MBCs initiated from selected corm slices of the banana cultivar ‘Rastali’ (AAB) were cultured on Murashige and Skoog medium supplemented with various concentrations of BAP hormone in both solid and liquid cultures. Optimisation was assessed based on the number and size of MBCs produced (>5 mm, 3-5 mm and <3 mm). The MBCs production was monitored at 2-week intervals for a period of 6 weeks. The highest number of single buds (32 to 36 buds) produced from MBCs was obtained with 10 mg/L of BAP in both solid and liquid medium.
Use of an Improved Site-Directed Mutagenesis for Creation of Constructs to Analyse Gene Function in *Fusarium oxysporum* f. sp. *cubense*


*Fruit Tree Research Institute, Guangdong Academy of Agricultural Sciences, Guangdong Province, China*

*Fusarium oxysporum* f. sp. *cubense* (Foc) is the causal pathogen of Fusarium wilt in banana. Apart from plant resistance, no sustainable solution has been found to control Fusarium wilt. Understanding the genetic basis of pathogenesis in Foc might contribute to developing novel strategies to control Fusarium wilt. A number of molecular tools have been developed to study pathogenicity in fungal phytopathogens, including *Fusarium* spp. Here, we used an improved site-directed mutagenesis to create constructs to analyse gene function in Foc. For the general site-directed mutagenesis, sequencing is the only choice to confirm the mutant clone; however in our protocol, named OE-PCR-S1, we not only improved the efficiency with Dpn I, but also used mismatch cleavage by S1 nuclease to replace sequencing to screen the mutants, which made the protocol more rapid and labour efficient and less expensive. We have used it for Foc, and have successfully deleted three bases from a pathogenic gene, *FOW1* (EU795421). The mutant DNA can be inserted into an expression vector and transformed into Foc for functional domain analysis. The overall rate of obtaining the mutant sites was 100%, and the whole mutagenesis process could be completed in less than 2 days, which means that the method is a powerful tool to study pathogenic gene functions and genetic engineering.
Development of Highly Regenerative Embryogenic Cell Suspensions and Transformants with AMP Gene of Banana Cultivar ‘Nanjangud Rasbale’ (syn. ‘Rasthali’, AAB, Silk subgroup)

S. Mohandas, H.D. Sowmya, R. Manjula, K.Y. Pratibha, M. Manamohan and S. Meenakshi

Division of Biotechnology, Indian Institute of Horticultural Research, Hessaraghatta, Bangalore 560 089, India

Banana cultivar ‘Nanjangud Rasbale’ (syn. ‘Rasthali’, AAB, Silk subgroup), one of the special banana cultivars of Karnataka State in India, is threatened by Fusarium wilt caused by Fusarium oxysporum f. sp. cubense (Foc). Genetic transformation techniques have shown great potential for inducing resistance to Foc. Regeneration through somatic embryos developed from embryogenic cell suspension (ECS) culture is one of the popular methods to transform banana. There is no report of the development of highly regenerative suspension cultures from this cultivar using floral meristems. Floral meristems were cultured on callus induction medium containing 2,4-D 4 mg/L. Embryogenic calli were selected and cell suspensions were developed and maintained on 2,4-D 1 mg/L. The cell suspensions plated on media containing BAP 1 mg/L and IAA 2 mg/L developed into embryos in 45 days and germinated in 60 days. One ml culture of the suspension produced 2080-2960 somatic embryos which produced 1500-2000 plantlets. Histological observations confirmed that 75% of the embryos were normal with shoot and root meristem, and 25 % showed various levels of abnormalities. After standardising different transformation conditions, the ECS (0.5OD) was cocultivated with Agrobacterium containing AMP gene in pCambia 2301 vector for 30 minutes with 100 µM acetosyringone and plated on 50 mg/ml G418. Somatic embryos developed and regenerated on same selection medium with a total germination of 12%. RT-PCR, Dot blot and Southern blot assay of the regenerants showed integration of the gene in 10 out of 17 plants tested. Further evaluation of the transgenics is in progress.
Improving Regeneration of Transformed Banana by Reducing Explant Browning

J.Y. Li¹, J.B. Zhang², B.Y. Xu² and Z.Q. Jin¹,²

¹HaiKou Experimental Station, Chinese Academy of Tropical Agricultural Sciences, Haikou 570102, China; ²Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou 571101, China

Low regeneration caused by explant browning in the progress of gene transformation into banana is a limitation in gene transfer. To decrease explant browning, the male flowers from banana cultivar ‘Baxi Jiao’ (AAA, Cavendish subgroup) were used as explants which were transformed by using Agrobacterium tumefaciens-mediated gene transformation. The results indicate that explant browning is reduced sharply in modified MS medium with NH₄⁺ and NO₃⁻ at a ratio of 20.6/67.6 (mol). The results also indicate that somatic embryogenesis was induced effectively from explants in MS medium containing 1.0 mg/L of 6-BA.

New Cultivars, New Options: The Potential of Introduced Bananas

F.S. dela Cruz, Jr.¹, L.S. Gueco¹, O.P. Damasco¹, V.C. Huelgas¹, F.M. dela Cueva¹, T.O. Dizon¹, M.L.J. Sison¹, V.G.O. Sinohin² and A.B. Molina²

¹National Plant Genetic Resources Laboratory, Crop Science Cluster, Institute of Plant Breeding, University of the Philippines Los Baños, College, Laguna 4031, Philippines; ²Commodities for Livelihoods programme, Bioversity International - Asia Pacific, 3F Khush Hall, IRRI, Los Banos, Laguna, Philippines

Performance of six FHIA hybrids and six local cultivars was evaluated in terms of agronomic and yield characteristics, visual and organoleptic (taste) acceptability, and reaction to common diseases at the experiment station of the Institute of Plant Breeding, University of the Philippines Los Baños, Philippines. Results showed that ‘FHIA-17’, ‘FHIA-23’ and ‘FHIA-25’ produced significantly heavier bunches than the highest yielding local cultivar ‘Cardaba’ (BBB), with ‘FHIA-17’ also producing the heaviest individual
fruits. However, these introduced cultivars were less sweet than the local cultivars based on total soluble solids (TSS) reading. Most of the introduced cultivars showed fewer symptoms from infection by *Banana bunchy top virus* (BBTV) and *Mycosphaerella fijiensis*, the causal agent of black leaf streak, than the popular local cultivar ‘Lakatan’ (AAA). ‘Saba’ (BBB) is the preferred cultivar for consumption as boiled fruit, while ‘Lakatan’ is more preferred than the introduced cultivars as dessert banana. However, ‘FHIA-01’ is better liked as cake compared with the local Cavendish type ‘Buñgulan’ (AAA). All FHIA hybrids (except ‘FHIA-25’) are preferred over the local ‘Saba’ for processing into honey-flavoured and salted chips. This highlights the cultivars’ potential as raw material for value-added processed products (banana chips) for both the local and export markets.

**Distribution of Commercial Cultivars, Landraces and Wild *Musa* in Indonesia**

A. Sutanto\(^1\), C. Hermanto\(^1\), H.S. Edison\(^1\), Jumjunidang\(^1\), A.B. Molina\(^2\), J. Daniells\(^3\) and W. O’Neill\(^4\)

\(^1\)Indonesian Tropical Fruit Research Institute, Jl. Raya Solok - Aripa km 08, PO Box 5, Solok 27301, West Sumatera, Indonesia; \(^2\)Commodities for Livelihoods programme, Bioversity International - Asia Pacific, 3F Khush Hall, IRRI, Los Banos, Laguna, Philippines; \(^3\)Department of Primary Industries and Fisheries, PO Box 20, South Johnstone 4859, Queensland, Australia; \(^4\)Department of Primary Industries and Fisheries, 80 Meiers Rd, Indooroopilly 4068, Queensland, Australia

Indonesia, being part of the centre of origin of *Musa*, has a large diversity of *Musa* species. Some cultivars are planted by farmers for domestic and commercial use, and some grow wild in the forest. Collecting missions of Musaceae have been carried out in most of parts of Indonesia in the past; however, some data did not have georeference information. The latest collecting mission of Musaceae was completed by the Indonesian Tropical Fruit Research Institute (ITFRI) through a project funded by the Australian Center for International Agricultural Research (ACIAR) and coordinated by Bioversity International. The survey and collecting missions were done in 15 provinces, and were in conjunction with the study on survey and characterisation of banana wilt diseases in Indonesia. The surveys showed that
commercial banana cultivars differ between provinces, but the most common commercial cooking cultivars are ‘Kepok’ (ABB/BBB, Saba), ‘Raja’ (AAB) and ‘Tanduk’ (AAB, Horn Plantain), while commercial dessert cultivars are mainly ‘Ambon Kuning’ (AAA, Gros Michel), ‘Ambon Hijau’ (AAA, Giant Cavendish), ‘Barangan’ (AAA), ‘Raja Serai’ (AAB, Silk) and ‘Mas’ (AA, Sucrrier). ‘Kepok’, ‘Raja’ and ‘Ambon Hijau’ can be found throughout Indonesia. Besides those commercial cultivars, landraces were found in some areas. Some of them are threatened by banana diseases and the development of commercial banana cultivation programmes. A landrace cultivar found in Sumatera, West Java and Nusa Tenggara Barat is ‘Pisang Ketan/Jantan/Uli/Ketip’, while ‘Uti Cere/Goroho’ can be found in North and South Sulawesi. ‘Pisang Jari Buaya’ (AA), ‘Lilin’ (AA) and ‘Sario’ (AA) are easily found in West Sumatera. Some wild Musa species may be found in the forest and open areas near the roads, waterfalls and valleys. Nasution (1991) had found fifteen varieties of Musa acuminata. Eight of these varieties have been rediscovered. They are M. acuminata var. halabanensis, M. acuminata var. acuminata, M. acuminata var. longipetiolata, M. acuminata var. zebrina, M. acuminata var. malaccensis, M. acuminata var. sumatrana, M. acuminata var. microcarpa and M. acuminata var. flava.

**Alternative Ex-Situ Conservation Strategy to Ensure Virus-Free Status and True-to-Typeness of Musa Germplasm**

A.B. Molina¹, F.S. Dela Cruz², L.S. Gueco², O.P. Damasco², V.C. Huelgas², F.M. dela Cueva², T.O. Dizon², M.L.J. Sison², V.G.O. Sinohin¹, M. Alforque³ and L.E. Herradura³

¹Commodities for Livelihoods programme, Bioversity International - Asia Pacific, 3F Khush Hall, IRRI, Los Banos, Laguna, Philippines; ²National Plant Genetic Resources Laboratory, Crop Science Cluster, Institute of Plant Breeding, University of the Philippines Los Baños, College, Laguna 4031, Philippines; ³Davao National Crop Research and Development Center, Bureau of Plant Industry, Tugbok District, Davao City, Philippines

Banana germplasm conservation is a real challenge in countries like the Philippines where diseases are prevalent. Musa accessions maintained in field genebanks are lost or become useless because of infection, particularly by
viruses. In-vitro conservation by subculturing meristem cultures under low temperature is fraught with risk of somaclonal variation and loss due to unreliable power supply, and has a high maintenance cost. The loss of the in-vitro duplicate of *Musa* collections at the Bureau of Plant Industry (BPI) in Davao a few years back is just one example. An alternative in-vivo conservation technique was evaluated at the Philippine National Repository, Multiplication and Dissemination Centres (NRMDCs), hosted by BPI and the Institute of Plant Breeding (IPB) and coordinated by Bioversity International. One of the aims of these NRMDCs was to introduce and maintain disease-free foundation stocks of improved, high-yielding and disease-free cultivars of banana, making them readily available to farmers, researchers and other interested individuals and institutions in the country. Twenty-two accessions from the International Transit Centre, Belgium, and seven local cultivars were established in the fields at BPI and IPB in 2002. Replicates of these accessions were maintained in-vitro and in-vivo planted in pots in an insect-proof screenhouse. The plants were maintained in plastic (IPB) or clay (BPI) pots, and when they reached a height of 3 m, they were cut 15 cm from the ground for regrowth. Monitoring for virus infection, specifically BBTV, was done in the field and the BPI screenhouse on the basis of symptomatology, while both symptomatology and ELISA tests were used in the screenhouse at IPB. After 1-2 years in the field, several accessions showed BBTV infection and were eliminated. In the screenhouse, no BBTV infection is noted after 4 years based on symptomatology and bi-annual BBTV ELISA tests (IPB only). This technique of in-vivo maintenance is now viewed as an alternative long-term conservation strategy to offset the limitations and risks of the traditional ex-situ conservation approaches of field conservation backed up by in-vitro strategies.

**Status of *Musa* Genetic Conservation in India**

H.P. Singh

*Indian Council of Agricultural Research, New Delhi, India*

Banana and plantain contribute significantly to the socio-economics of India and provide livelihood to millions of people. Over the years, banana research has received due attention in the country, with an institutional mechanism for basic and strategic research, and programmes for the generation of location-
and situation-specific technologies. This has resulted in increased production, productivity and availability of banana, and India is now the largest producer and consumer of banana in the world. Technology adoptions include the use of improved cultivars, technology-led production systems management, nutrition, water productivity, in-vitro propagated plants, plant health management and improved marketing. Being a major centre of diversity for *Musa*, efforts have been made for prospection, collection, conservation, characterisation, documentation and evaluation of genetic resources. Past collection efforts of *Musa acuminata* ssp. *burmanicoides* (‘Calcutta 4’) have contributed significantly to the gene source for resistance to black leaf streak in global programmes, and many accessions with genes for resistance to biotic and abiotic stresses and for other desirable traits were identified. The Indian *Musa* germplasm repository at the National Research Centre on Banana, Trichy comprises 942 accessions, including an indigenous wild species, more than 15 landraces and 50 unique accessions. Many of the accessions are safely duplicated at different locations with distinct agroclimatic conditions, which has enabled to understand interactions between genotype and environment. The accessions are also maintained in vitro for medium-term conservation, and cryo-preservation is used for long-term conservation. Names and synonyms of Indian banana have been documented in a catalogue. The *Musa* Germplasm Information System (MGIS) is being used for developing a database. Accessions have also been characterised using molecular markers. Some cultivars, like ‘Virupakshi’ (AAB) and ‘Nanjangud Rasbale’ (AAB), have been registered under GI for their uniqueness. Evaluation in field genebanks for biotic and abiotic stresses has resulted in the identification of resistant gene sources for nematodes, Fusarium wilt, Sigatoka leaf spot, virus diseases and drought. Breeding work started in the early 1960s has resulted in the development of some improved hybrids, and many synthetic diploids, which are further being used in improvement programmes. An effective transformation and regeneration protocol has also been developed, and transformants are in the process of evaluation.
In-Vitro Conservation and Cryopreservation of Genetic Resources of *Musa* in India: A Network Approach

A. Agrawal, R.K. Tyagi and S.K. Sharma

*National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi 110 012, India*

Banana and plantain (*Musa* spp.) are a major staple food and fruit crop in India. Being one of the centres of origin and diversity for *Musa*, India is endowed with a rich polyclonal diversity of cultivated germplasm. Additionally, several endemic wild species like *Musa andamanica, Musa cheesmannii, Musa nagensium* and *Musa sikkimensis* are present. Threats posed by habitat destruction, diseases and pests, and replacement or loss of traditional cultivars necessitate the need to collect and conserve germplasm of *Musa*. The National Bureau of Plant Genetic Resources (NBPGR), New Delhi was mandated to carry out in-vitro conservation of *Musa* since 1986, to complement the field conservation programmes in other institutes. Germplasm was collected in the form of suckers from several field genebanks, and in-vitro cultures were established using standard protocols. Currently, about 410 accessions of *Musa* are conserved in vitro, the storage period varying from 6 to 16 months depending on the cultivar. The in-vitro collection consists of both indigenous (70%) and exotic (30%) accessions. All the germplasm collections are documented with minimum passport data, and about 70% genotypes have been characterised and/or evaluated. Research on cryopreservation of *Musa* by vitrification of shoot meristems (proliferating meristems and individual meristems) was initiated in 2001. Three different methods of cryopreservation (simple freezing, vitrification and droplet freezing) were tested on 15 genotypes. Cryopreservation of the proliferating meristems using the vitrification technique yielded 20-67% post-thaw regrowth, whereas use of droplet freezing yielded higher regrowth (40-85%). Genetic stability testing of nine cryopreserved accessions was carried out using morphological and molecular markers (STMS). No significant variation has been observed between the control and cryopreserved plants, till date. Thus, cryobanking of 13 accessions of *Musa* belonging to ABB, AAB, AAA and AB genome groups has been carried out.
Conservation, Evaluation and Utilisation of Introduced *Musa* Germplasm in Kerala, India

R. Menon, A. Cherian, A. Suma and S. Nair

*Banana Research Station, Kerala Agricultural University, Kannara, Thrissur 680652, Kerala, India*

The Banana Research Station (BRS), Kannara has introduced 50 exotic *Musa* germplasm accessions, with the aim to evaluate the material, use it in a breeding programme and make available selected superior material to the farmers of Kerala. The material was obtained from the *Musa* collection at the International Transit Centre, Belgium. The accessions were multiplied and supplied by NBPGR, New Delhi in the form of small suckers or proliferating in-vitro cultures. The accessions comprised 22 reference cultivars, 6 wild species and 22 improved hybrids/variants. They were regenerated to plantlets and field established for evaluation at BRS. Selected AA wild/edible diploids with disease resistance are included in breeding work as pollen sources. Plantain-derived diploid hybrids, ‘TMP2x 2829-62’ and ‘TMP2x 1297-3’ from IITA, Nigeria are used for hybridisation with local plantain cultivars. False horn plantain ‘Big Ebanga’ (AAB) showed good adaptability and bunch yield in on-farm evaluation. ‘Yangambi Km5’ (AAA), a cultivar with excellent establishment, has been popularised as a dessert banana with acceptable bunch qualities and resistance to major fungal diseases and insect pests. Fourteen improved hybrids were evaluated for growth and yield parameters over two seasons under Phase II and III of the International *Musa* Testing Programme (IMTP) in association with NRCB, Trichy. The evaluation site at BRS, located at an elevation of 58 m has an average temperature of 28°C, annual rainfall of 3000 mm and relative humidity between 77 and 95%. Infection index based on Gauhl’s modification of Stover’s severity scoring system and the youngest leaf spotted were used to evaluate the resistance of hybrids to Sigatoka leaf spot. The hybrids ‘FHIA-01’, ‘FHIA-03’, ‘SH-3640’, ‘FHIA-18’, ‘FHIA-21’, ‘FHIA-25’, ‘CRPB-39’ and ‘TMB 5295-1’ registered superior bunch features. Bunch weight ranged from 16 kg in ‘FHIA-18’ to 37 kg in ‘FHIA-25’. The hybrids showed very high resistance to Sigatoka leaf spot. ‘SH-3640’ and ‘FHIA-03’ are currently under multilocational testing and are well accepted by smallscale farmers.
Evaluation and Tissue-Culture Conservation, Multiplication and Distribution of Rare and Carotenoid-Rich Fe’i Banana Cultivars in Micronesia

V.M. Verma

_Micronesia Plant Propagation Research Center, Agricultural Experiment Station and Cooperative Extension, College of Micronesia-FSM, Kosrae FM 96944, Micronesia_

_Musa_, a plant genus of extraordinary significance to human societies, produces the fourth most important food in the world today, the edible banana and plantain. Banana is one of the most important fruit crops grown in the Micronesian Region for local consumption as well as for export. Two cultivars of rare Fe’i banana, ‘Kulasr’ and ‘Kulondol’, have yellow and orange flesh and are rich in provitamin A and other carotenoids. Both are robust plants bearing erect bunches of brilliant orange-gold to purple fruits at maturity, which are delicious and nutritious. Tissue-culture multiplication of banana provides excellent advantages over traditional multiplication, including a high multiplication rate, physiological uniformity and the availability of disease-free material throughout the year, ensuring safe and rapid distribution of new planting materials. A study was undertaken to develop an efficient, rapid and reproducible regeneration protocol for ‘Kulasr’ and ‘Kulondol’ for mass multiplication and in-vitro conservation. Banana plantlets were produced at mass scale through tissue culture, acclimatised in the greenhouse, distributed and established in the field. Ultimately, this exercise helped greatly to save rare Fe’i bananas on Micronesian islands. This paper discusses the research and extension work done on evaluation and tissue-culture conservation, multiplication and distribution of these two rare and carotenoid-rich Fe’i banana cultivars on small islands of Micronesia.

Micropropagation of Malaysian Banana Cultivars (Musa spp.) Using a Modified Twin-Flask System

L.K. Chan and V.H. Au

_Plant Tissue and Cell Culture Laboratory, School of Biological sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia_

In Malaysia, banana is one of the most important food crops. The commercial planting of Malaysian banana cultivars has resulted in a demand of planting
materials which is greater than the supply. In-vitro propagation of banana using the conventional gel medium was found to be slow and labour intensive. Our study showed that micropropagation using liquid-culture medium in a modified aerated twin-flask system could increase the number of shoots formed from each shoot explant. Dividing the shoot explants longitudinally and culturing them in the twin-flask system could further enhance the formation of multiple shoots. The optimum conditions for obtaining the highest number of multiple shoots were evaluated using the modified twin-flask system. Four half-shoot explants, derived from in-vitro plantlets, cultured in 100 ml shoot proliferation medium in 500 ml capacity flasks gave the best results. The medium was supplied to the explants for 20 minutes twice a day. The addition of 30 g/L sucrose into the proliferation medium maintained at pH 5.7 could induce 6-7 shoots from each of the half-shoot explants after 5 weeks of culture in the presence of light with intensity of 2000-2500 Lux. The plantlets were acclimatised by transferring them into plastic trays containing organic soil : top soil : sand mixture (1:1:1) after rinsing the rooted plantlets under running water for 15 minutes to remove any traces of the culture medium. The trays were covered with plastic sheets with small punched holes and placed under shaded greenhouse for 2 weeks. All the rooted plantlets had a height of more than 5 cm and survived after acclimatisation. They were transferred to polybags containing the same soil mixture and placed in the greenhouse for another 2 weeks. The 1-month old seedlings were then ready for transplanting to the field.

Cryopreservation of In-Vitro Shoot Tips of *Musa* Germplasm by Droplet Vitrification

J.G. Li$^{1,2}$, S.M. Zhang$^1$, H.B. Chen$^1$, C.X. Xu$^1$ and Z.H. Wang$^1$

$^1$Tropical and Subtropical Laboratory, South China Agricultural University, Guangzhou, China; $^2$Institute of Biotechnology on Horticultural Crop, South China Agricultural University, Guangzhou, China

Experiments were conducted to establish an improved protocol for the cryopreservation of banana (*Musa* spp.). The droplet vitrification method for shoot tips of AAA and ABB genotypes was optimised using an orthogonal time table for loading, dehydration and recovery. The effects of different
cryoprotectants and shoot-tip types on cryopreservation were also studied. The best procedure was as follows: a shoot tip 1.5-2.0 mm in length and partially covered by 1-2 leaf primordia was excised and pretreated with a loading solution for 120 min at room temperature. After loading, the solution was replaced by ice-cooled PVS2 for 30-50 min. Each shoot tip was then transferred to a droplet of PVS2 solution on a strip of aluminium foil and plunged into liquid nitrogen directly. After 1 h, the strips were rinsed in unloading solution at room temperature for 15 min. The shoot tips were placed onto a piece of sterilised filter paper on top of semisolid MS medium containing 0.3 mol/L sucrose. After 24 h, the shoot tips were transferred onto regeneration medium without filter paper. Using this droplet vitrification protocol, we successfully cryopreserved 15 *Musa* accessions belonging to five different genomic groups. The average survival rate was 78.0% for AAA group, 57.8% for AAB group, 72.1% for ABB group, 43.3% for AA group and 42.2% for *M. itinerans*. This optimal droplet vitrification procedure can be used for routine cryopreservation of *Musa* germplasm.

**Effect of Cytokinins on the Proliferation Rate of Banana (Musa spp.) Plantlets**

M. Maziah$^{1,2}$, F. Mahdavi$^2$, M. Sariah$^2$, M.P. Puad$^3$ and S. Shirani$^2$

$^1$Department of Biochemistry, Faculty of Biotechnology and Molecular Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor DarulEhsan, Malaysia; $^2$Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor DarulEhsan, Malaysia; $^3$Department of Cell and Molecular Biology, Universiti Putra Malaysia, 43400 Serdang, Selangor DarulEhsan, Malaysia

Banana and plantain (*Musa* spp.) are major staple crops for millions of people in the tropics and their export contributes to the economies of many countries. But, in recent years, their production has been seriously threatened by stress factors, such as decreasing soil fertility and diseases. Use of in-vitro techniques to produce large numbers of disease-free plantlets have been continuously researched and developed. In this study, different cytokinins are evaluated in an effort to further increase the proliferation rate of in-vitro banana plantlets. Rhizome slices of ‘Nangka’ (AAB) and ‘Baka Baling’ (ABB) were cultured onto Murashige and Skoog (MS) medium supplemented with 1 mg/L of
thidiazuron (TDZ), 6-benzylaminopurine (BAP), kinetin (Kin), 2-isopentenyl adenine (2-ip) and zeatin (Zea) to determine the best cytokinin for obtaining a high proliferation rate. The results obtained showed that TDZ produced 8.5 and 7.6 shoots per explant and BAP produced 5.5 and 4.8 shoots per explant, for ‘Baka Baling’ and ‘Nangka’, respectively. Meanwhile, kin, 2-ip and Zea each produced 3.4, 1.6 and 1.2 shoots per explant. Subsequent experiments using different concentrations of TDZ (0, 0.2, 0.4, 0.6, 0.8 and 1 mg/L) and BAP (0, 2, 4, 6, 8, 10, 12, 14 and 16 mg/L) were carried out. TDZ at 0.8 mg/l resulted in 17.6 and 13.3 shoots per explant for ‘Nangka’ and ‘Baka Baling’, respectively. BAP at 8 mg/L was determined as the best concentration producing up to 18.2 and 15.4 shoots per explant in ‘Baka Baling’ and ‘Nangka’, respectively, after 8 weeks of culture. Rooting was observed in both cultivars in treatments containing 0.4 mg/L of TDZ. The results showed that TDZ can be considered as an alternative cytokinin for commercial micropropagation of banana cultivars.

The Effect of Different Cytokinins on Plant Regeneration from Male Flowers of Banana

F. Mahdavi\textsuperscript{1}, M. Maziah\textsuperscript{2}, M. Sariah\textsuperscript{1}, M.P. Puad\textsuperscript{3} and S. Shirani\textsuperscript{1}

\textsuperscript{1}Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor DarulEhsan, Malaysia; \textsuperscript{2}Department of Biochemistry, Faculty of Biotechnology and Molecular Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor DarulEhsan, Malaysia; \textsuperscript{3}Department of Cell and Molecular Biology, Universiti Putra Malaysia, 43400 Serdang, Selangor DarulEhsan, Malaysia

Banana and plantain (\textit{Musa} spp.) are an important food crop, especially in Southeast Asia. Today, their production is seriously threatened by Fusarium wilt. Micropropagation can play a significant role in increasing yield and producing disease-free plants. Different explants have been used for banana and plantain propagation, and the use of shoot tips for in-vitro propagation of bananas is common for many of the commercial cultivars. Male flowers have the potential to be a useful source of explants for micropropagation. The major objective of this study was to develop a plant regeneration protocol for banana using the male flowers as explants. Male flowers of ‘Nangka’ (AAB), ‘Rastali’ (AAB) and ‘Berangan’ (AAA) were cultured onto Murashige and Skoog (MS)
medium supplemented with 1 mg/L of thidiazuron (TDZ), 6-benzylaminopurine (BAP), kinetin (Kin), 2-isopentenyl adenine (2-ip) and zeatin (Zea) to obtain the best cytokinin for plant regeneration. The results obtained showed that TDZ produced 9.4, 8.75 and 8 shoots per explant for ‘Berangan’, ‘Nangka’ and ‘Rastali’, respectively, after 2 months of culture. BAP produced 5.4, 5 and 4.5 shoots per explant in the respective cultivars, ‘Berangan’, ‘Nangka’ and ‘Rastali’. In a separate experiment, male flowers were cultured onto MS medium with different concentrations of TDZ (0, 0.2, 0.4, 0.6, 0.8, 1 mg/L) and BAP (0, 2, 4, 6, 8, 10, 12, 14 mg/L). It was observed that 0.4 mg/L of TDZ was the optimum concentration for proliferation rate (0.64) of male flower in ‘Berangan’, while 0.6 mg/L was the best for ‘Nangka’ (0.52) and ‘Rastali’ (0.6). The results show that male flowers can be used as explants for micropropagation of bananas with reasonable proliferation capacity.

Effects of Cytokinins on Proliferation Rate and Abnormality Index of Banana (Musa spp.) Cultivars from Excised Shoot Tips

S. Shirani¹, M. Maziah¹,², M. Sariah¹, W.B. Zakaria³ and F. Mahdavi¹

¹Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor DarulEhsan, Malaysia; ²Department of Biochemistry, Faculty of Biotechnology and Molecular Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor DarulEhsan, Malaysia; ³Department of Crop Science, Universiti Putra Malaysia, 43400 Serdang, Selangor DarulEhsan, Malaysia

Banana provides a staple food for millions of people in the developing world. However, the crop is threatened by several abiotic stresses, such as drought. Shoot tips with high proliferating capacity can be used for mass clonal propagation which can be the preferred target material for induced mutations with the aim of developing tolerant varieties. The effect of cytokinins on the micropropagation rate in relation with frequency of abnormal shoot regeneration of banana cultivars ‘Berangan Intan’ (AAA), ‘Berangan’ (AAA) and ‘Rastali’ (AAB) were studied by culturing shoot tips (5-7 mm) on Murashige and Skoog (MS) medium supplemented with various concentrations of benzylaminopurine (BAP) and kinetin (Kin) (0.0, 11.1, 22.2, 33.3 and 44.4
µM) and thidiazuron (TDZ) (0.0, 0.5, 2, 5, 7.5 µM). Proliferated shoot tips were categorised as normal or abnormal based on morphological appearance. Evaluations were carried out by counting the abnormality index, and measuring the proliferation rate, shoot length and fresh weight. The results showed that shoot proliferation and abnormality index were significantly dependent on cytokinin type, its concentration and the banana cultivar. BAP below 22.2 µM did not improve shoot regeneration and at 33.3 µM significantly caused higher abnormal shoot production than 22.2 µM. Kinetin induced a lower number of shoots than that obtained with similar levels of BAP. TDZ at 5 µM stimulated multiplication of shoots (6.22, 6.17 and 14.17 per explant in ‘Berangan Intan’, ‘Berangan’ and ‘Rastali’, respectively), compared to Kin and BAP. However, it resulted in a high abnormality index (0.57, 0.67 and 0.82 for ‘Berangan Intan’, ‘Berangan’ and ‘Rastali’, respectively), rendering this treatment unusable. The length of shoots and fresh weight in proliferated shoot tips significantly decreased with increasing concentration of cytokinins. The results thus demonstrated that optimal shoot proliferation rates (3.44, 4.22 and 7.67 shoots per explant for ‘Berangan Intan’, ‘Berangan’ and ‘Rastali’, respectively) were achieved with BAP at 22.2 µM. Consequently, this optimised proliferating system can be used for inducing mutation using shoot-tip culture.

The Effect of Ethyl Methane Sulphomate (EMS) and Sodium Azide (NaN₃) on Plant Regeneration Capacity of Banana Cultivar ‘Yueyoukang 1’ (AAA), Highly Resistant to Fusarium Wilt

C.X. Xu, J. Xiao, J.G. He, G.B. Hu and H.B. Chen

College of Horticulture, South China Agricultural University Guangzhou 510642, China

The best approach to control Fusarium wilt of banana (Musa spp.) is to grow resistant cultivars. ‘Yueyoukang 1’ (AAA; Cavendish subgroup) is a cultivar highly resistant to Fusarium oxysporum f. sp. cubense race 4, but its yield is lower and its growth cycle longer in comparison with other commercial cultivars. In the present study, an embryogenic cell suspension (ECS) of this
cultivar was mutated with chemical mutagenic agents sodium azide (NaN$_3$) and ethyl methane sulphomate (EMS), followed by plant regeneration via somatic embryogenesis to optimise their dosage. The effects of treatment duration of EMS (0.2%, v/v) and the concentration of NaN$_3$ (treatment duration was 30 min) on the plant regeneration capacity of the ECS were studied. The plant regeneration capacity of the ECS decreased with the increase of the concentration of NaN$_3$. When the concentration of NaN$_3$ was 5.42 mmol/L, the plant regeneration capacity of treated ECS was 50% of that obtained in the control (LD$_{50}$ of NaN$_3$=5.42 mmol/L). The effect of EMS treatment on the plant regeneration capacity of ECS was also remarkable. When the ECS was treated with 0.2% EMS for 10 minutes, the plant regeneration capacity was even higher than that of the control. But the plant regeneration capacity decreased with increasing treatment duration. The amount of plants obtained per gram of ECS treated with 0.2% of EMS for 18.1 minutes was 50% of that obtained in the control (LD$_{50}$ of EMS=18.1 min). The dosages at LD$_{50}$ are suggested to be used for mutants with improved yield and growing cycle.

State of the Culture of the Banana Tree in a Mediterranean Zone: The Tunisian Coastal Oases of Gabes

M. Ben Salah$^1$, A. Ferchichi$^2$ and M. Jeridi$^2$

$^1$Arid Land Institute, Nahal 6051, Gabes, Tunisia; $^2$Arid Land Institute, El Fje, Medenine 4119, Tunisia

Banana (Musa spp.) are grown in coastal oases in Tunisia, located near the Mediterranean Sea in the South-East of the country. The arid climate is characterised by high temperatures in summer and moderate temperatures in winter (ranging from 5 to 25°C), and high evapotranspiration (1500 mm/year). The typical soil in oases is loam-sandy soil with high organic matter concentration in arid lands. To preserve the oases’ diverse fruit species, actions are undertaken to conserve and further develop banana trees and other fruit trees in those oases. Six locally grown cultivars have been described: ‘Arbi1’ (AAA), ‘Arbi2’ (AAA), ‘Guebsi Jwaid’ (ABB), ‘Guebsi Khchin’ (ABB), ‘Spani’ (ABB) and ‘Lobnani’ (ABB). Morphological description of the pseudostem, suckers, leaves, inflorescence, bracts, male flower and fruits was performed. Pomological descriptors included fruit weight, fruit dimensions,
number of fruits by hand, shape and transverse section of the fruit, apex, pulp, skin and pulp colours at maturity. The morphological data were collected and analysed by ACP method. The cultivars were grouped into two clusters: ‘Arbi1’ and ‘Arbi2’ in one group, and ‘Spani’, ‘Lobnani’, ‘Guebsi Kchin’ and ‘Guebsi Jwaid’ in another group. These two groups were especially distinguished by the fruit weight, diameter and length. All studied oasis cultivars are actually collected in experimental plots at the Arid Land Institute to further evaluate their agronomic features and production potential.

Evaluation of Some Cavendish Cultivars (Musa spp., AAA) under Plastic Greenhouses in Subtropical Areas of Turkey

H. Gubbuk¹, F. Bakry² and Y. Mathieu³

¹Department of Horticulture, Faculty of Agriculture, Akdeniz University, TR-07059 Antalya, Turkey; ²CIRAD, Genetic Improvement of Vegetatively Propagated Crops, TA A-75/02, Avenue Agropolis, 34398 Montpellier Cedex 5, France; ³VITROPIC S.A. ZAE des Avants, 34270 Saint Mathieu de Trèvières, France

Banana has been grown in Turkey for over a century as an open-field crop until the 1990s. In the last decades, the protected cultivation of banana gained significant commercial interest. The present investigation was undertaken to evaluate the potential of the Cavendish (AAA) cultivars ‘Williams’, ‘MA 13’ ‘Jobo’ and ‘CV 902’ as an alternative to ‘Grand Nain’ grown under subtropical conditions. The study was carried out in Bozyazi in the Mersin province of Turkey during the years 2006 and 2007. The yield and quality parameters were evaluated under naturally ventilated plastic greenhouses. The average minimum and maximum temperatures in the experimental site were 15/28°C, and the relative humidity averaged over 70%. Spacing was 3.0 x 1.8 m (1850 plants/ha) and a line drip irrigation system was installed on both sides (about 30-40 cm from the plants). Stem circumference, stem height, total leaf number, bunch stalk circumference, days from shooting to harvest, number of hands per bunch, number of fingers per bunch, finger circumference, finger length and bunch weight were measured. Cultivars varied among themselves with respect to the finger circumference (11.6-12.00 cm) and finger length (21.33-23.93 cm). ‘Williams’ had the highest bunch weight (51.93 kg), followed by ‘MA 13’ (51.83 kg). The lowest bunch weight was found in ‘Grand Nain’ (44.20 kg). Williams’, ‘MA 13’ and ‘CV 902 were agronomically superior to ‘Jobo’ and ‘Grand Nain’.
Monitoring Fusarium Wilt Race 4 in Hainan Province and Populations of *Fusarium oxysporum f. sp. cubense*

X. Zhang, H. Zhang, Y. Xie, J. Pu, Y. Qi and Y. Lu

*Key Laboratory of Monitoring and Control of Tropical Agricultural and Forest Invasive Alien Pests for Ministry of Agriculture, Environment and Plant Protection Institute, CATAS, Danzhou, China*

In 2008, according to the Banana Wilt Disease Monitor Technic Regulation, the status of Fusarium wilt on banana, caused by *Fusarium oxysporum f. sp. cubense* (Foc) race 4, in Hainan Province was monitored by Centers of Tropical Crops Technic Services for various cities and counties. The disease was found in 14 out of 18 cities and counties, namely Lingao County, Ledong County, Sanya City, Dongfang City, Chengmai County, Wenchang City, Wanning City, Baoting County, Quanzhou County, Tunchang County, Changjiang County, Baisha County, Haikou City and Danzhou City. The monitored area was 687.4 hectares, out of which 53.8 hectares were diseased with a total of 33,268 infected banana plants. The disease affected ‘Baxijiao’ (AAA), ‘Williams’ (AAA), ‘Fenjiao’ (ABB) and ‘Gaogangongjiao’ (AA). Diseased plants were eradicated and replaced by alternative crops or disease-resistant cultivars, like ‘Aigangongjiao’, ‘Nongke No. 1’, ‘Guangfen No. 2’ and others, as a control measure. Physiological race, pathogenicity, random amplified polymorphic DNA (RAPD) and vegetative compatibility group were determined for 18 Foc strains from Hainan Province and Guangdong Province. Pathogenicity test using different cultivars of ‘Baxijiao’ and ‘Fenjiao’, and nursery evaluation confirmed that twelve Foc strains isolated from ‘Fenjiao’ belonged to race 1, and six Foc strains from ‘Baxijiao’ were race 4. The pathogenicity varied.
among strains. RAPD analysis could differentiate geographic origins of strains of race 1 and race 4, approximately distinguish strains of race 1 and race 4 and distinguish pathogenicity grades of strains of race 4. The twelve race 1 strains isolated from ‘Fenjiao’ were assigned to VCG 1, and the six race 4 strains isolated from ‘Xiangyajiao’ were assigned to VCG 2, showing that vegetative compatibility groups of Foc strains are related to host origin and race. (Note: VCG 1 and 2 were designed by the research team because vegetative compatibility testers mentioned in the published literature were not available).

Risk Analysis on Introduction of Banana Fusarium wilt to Yunnan

L. Zeng, X.D. Li, H.C. Fan and Z.X. Guo

Institute of Agriculture Environment and Resource, Yunnan Academy of Agricultural Sciences, Kunming 650205, China

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *cubense*, is a devastating disease of banana globally. It was introduced in Guangdong, Hainan, Guangxi, Fujian and Taiwan provinces and has caused serious damage to banana production in China in recent years. Based on the production status of banana in Yunnan, a brief introduction, including the origin, geographical range, biological/ecological characteristics and spread of the pathogen, and the challenges of prevention and control will be given in this paper.

Status of Fusarium Wilt in India

H.P. Singh

Indian Council of Agricultural Research, New Delhi, India

In India, banana is affected by a large number of diseases and pests, with their severity depending upon the cultivars, agroclimatic conditions and production system management. Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* (Foc) is one of the most devastating diseases. The Silk subgroup (AAB) is seriously affected, but other cultivars in the group of Pisang Awak
(ABB), Ney Poovan (AB) and Bluggoe (ABB) also get infected, though the intensity of damage may vary. The pathogen can be classified using vegetative compatibility grouping (VCG). In India, isolates belonging to VCG 0124, 0124/0124/0125, 0128 and 01222 have been identified. Investigations indicate that Fusarium wilt becomes even more devastating in the presence of nematodes. Management practices adopted include disinfection of soil using solarisation, use of disease-free planting material, use of more organic matter and also use of *Bacillus subtilis*, *Pseudomonas fluorescens* and *Trichoderma harzianum*. These management practices are effective for one or two cropping cycles, but cannot manage the disease after more crop cycles. In this presentation, lessons learnt from the past and guidelines for the future for dealing with emerging issues in banana plant health will be discussed.

**First Report on the Occurrence of a Virulent Strain of**

*Fusarium oxysporum* f. sp. *cubense* VCG 0124 (Race 1)

**Infecting Cavendish (AAA) Banana**

R. Thangavelu, M. Gopi and M.M. Mustaffa

*National Research Centre for Banana (NRCB-ICAR), Thogamalai Road, Thayanur (Post), Tiruchirapalli 620102, Tamil Nadu, India*

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *cubense* (Foc), is one of the most serious and devastating diseases of banana worldwide, including in India. Four races have been reported in Foc, but only race 1 and 2 have been identified in India so far. These two races infect most of the commercial cultivars grown in India, except those belonging to the Cavendish subgroup (AAA). Cavendish cultivars are reported to be susceptible only to race 4 of Foc. Recently, Fusarium wilt was observed in Cavendish in India, and the pathogen isolated was identified as Foc based on cultural and morphological characters. Further characterisation of the pathogen by vegetative compatibility group (VCG) analysis and volatile odour production indicated that the pathogen belongs to VCG 0124 (race 1) and does not produce volatile aldehydes (“inodoratum” group). A pot experiment confirmed that this particular strain is highly virulent as it caused a maximum disease score of 6 in the majority of the
commercial cultivars grown in India. This is the first report on the prevalence of a new strain of Foc race 1 (VCG 0124) infecting Cavendish cultivars.

**Banana Cultivars and *Fusarium oxysporum* f. sp. *cubense* in Indonesia – Observations from Fusarium Wilt Disease Databases**

J.W. Daniells¹, W. O’Neill², C. Hermanto³ and R.C. Ploetz⁴

¹Queensland Primary Industries & Fisheries, South Johnstone, Queensland, Australia; ²Queensland Primary Industries and Fisheries, 80 Meiers Rd, Indooroopilly, 4068, Queensland, Australia; ³Indonesian Tropical Fruit Research Institute, PO Box 5, Solok 27301, West Sumatra, Indonesia; ⁴University of Florida, 18905 SW 280th Street, Homestead, Florida 33031-3314, USA

Genetically related populations of *Fusarium oxysporum* f. sp. *cubense* (Foc) are defined by vegetative compatibility groups (VCGs). In 2006, Queensland Primary Industries & Fisheries (QPIF), the Indonesian government and Bioversity International commenced an ACIAR project which included a study of the geographical spread of Foc in Indonesia. The project also looked at known information on relationships between banana cultivars and Foc VCGs, by consulting two databases that existed for Foc disease samples going back to 1992 and maintained by QPIF and the University of Florida in Homestead. Information from the project and these databases suggested that: (i) Most cultivars are susceptible to VCG 01213 (tropical race 4); (ii) ‘Gros Michel’ (AAA), ‘Silk’ (AAB) and ‘Saba’ (ABB) are each susceptible to several VCGs; (iii) Some cultivars tend to have certain VCGs associated with them; and (iv) ‘Sucrier’ (AA), ‘Lakatan’ (AAA) and ‘Mysore’ (AAB) are only affected by VCG 01213. Correct identification of cultivars from which Foc had been collected may have been an issue pre-project. Photos of cultivars should accompany Foc samples for identification. Although a greater understanding of these relationships would require pathogenicity studies with representative isolates of the different VCGs, these observations reiterate prior concerns over VCG 01213 and its continued spread in Asia.
A Molecular Detection Method Specific to *Fusarium oxysporum* f. sp. *cubense* Race 4 in Taiwan

P.F.L. Chang\(^1\), Y.H. Lin\(^1\), J.Y. Chang\(^1\), C.P. Chao\(^2\) and J.W. Huang\(^1\)

\(^1\)Department of Plant Pathology, National Chung Hsing University, Taichung City, Taiwan 402, Republic of China; \(^2\)Taiwan Banana Research Institute, Pingtung, Taiwan 904, Republic of China

Fusarium wilt is one of the most serious fungal diseases in banana (*Musa* spp.) and one of the major limiting factors for banana production worldwide. This disease is caused by *Fusarium oxysporum* f. sp. *cubense* (Foc). A rapid and reliable diagnosis and pathogen detection is the key to control Fusarium wilt of banana. Therefore, we have developed a reliable PCR technique to detect Foc race 4 isolates in Taiwan. The primer set derived from the sequence of a random primer OP-A02 amplified fragment produced a 242 bp DNA fragment which was specific to Foc race 4. This molecular method was sensitive and could detect as low as 10 pg of Foc DNA in 50 to 2000 ng host genomic DNA without affecting the amplification efficiency. We also demonstrated that the Foc pathogen could be easily differentiated from other formae speciales of *F. oxysporum* by using our PCR assay. In addition, in a recent survey, we found some Foc isolates in Taiwan that should be defined as tropical race 4 (TR4, VCG1213/16 as reported by R.C. Ploetz) according to the VCG test, but the current PCR method could not differentiate TR4 from subtropical race 4 isolates in our collection. This work was supported in part by BAPHIQ, and DIA, Council of Agriculture, Executive Yuan, Taiwan, R.O.C. under grant numbers 89ST-6.2-BQ-65(06), 90AS-6.3.1-BQ-B2(6), 91AS-7.3.1-BQ-B2(3), 93AS-1.9.2-BQ-B1(1), 96AS-4.1.2-IC-I1(2), 97AS-4.1.2-IC-I1(6) and 98AS-4.1.1-IC-I1(1); by the Ministry of Education, Taiwan, R.O.C. under the ATU plan; and by the National Chung Hsing University, Taiwan, R.O.C.
Cloning and Diversity Analysis of FGA1 from Two Fusarium oxysporum formae speciales


Fruit Tree Research Institute, Guangdong Academy of Agricultural Sciences, Guangdong Province, China

Fusarium oxysporum f. sp. cubense (Foc) is the causal agent of Fusarium wilt of banana, with race 1 infecting Fenjiao cultivars, and race 4 infecting Fenjiao and also Cavendish cultivars. Basing on the published genomic sequences of F. oxysporum f. sp. radicis-lycopersici, an important pathogenicity gene, FGA1, was isolated using the method of homology-based cloning. Sequences of Foc race 1 and Foc race 4 from different positions were aligned. The results showed that the full length of gDNA of Foc race 1 and Foc race 4 from Hainan is 1286 bp, and their sequence homology is 100%. The sequence of Foc race 1 FGA1 was conserved, including 4 exons and 3 introns, which encoded a protein with 353 amino acids. However, 80% of Foc race 4 FGA1 had two transcripts, with the smaller one similar to Foc race 1 FGA1 and the larger one encoding a polypeptide with 372 amino acids due to an alternative splicing making the third intron become an exon. FGA1 is the α-subunit of G-protein, and phylogenetic studies indicated that this protein was much conserved. The correlation between the alternative splicing of FGA1 gene and the variation of the virulence or hosts need to be further investigated.

Comparison of Three Inoculation Techniques for Pathogenicity Tests on Fusarium wilt of Banana

C.E. Soguilon¹, L.E. Herradura¹, A.G. Yebes¹, V.O. Sinohin² and A.B. Molina²

¹Davao National Crop Research and Development Center, Bureau of Plant Industry, Tugbok District, Davao City, Philippines; ²Commodities for Livelihoods programme, Bioversity International - Asia Pacific, 3F Khush Hall, IRRI, Los Banos, Laguna, Philippines

The recent confirmation of the occurrence of tropical race 4 of Fusarium oxysporum f. sp. cubense (Foc TR4) in the Philippines is of great concern to the
banana industry. Understanding the virulence of the various VCG’s and/or Foc isolates relative to the susceptibility/resistance of various banana cultivars is one research area of much interest. A reliable and accurate screenhouse inoculation and assessment protocol is needed. This study was conducted to compare three inoculation techniques, namely: (1) root-dipping technique as described by Mohamed et al. (1999); (2) spore-pouring technique by Chao (2006); and (3) corn meal-sand inoculum technique by Magnaye et al. (1969). Three cultivars, namely ‘Cavendish’ (AAA), ‘Lakatan’ (AAA) and ‘Latundan’ (AAB), were inoculated using Foc isolates obtained from field-infected Cavendish plants and previously identified to belong to VCG1213/16. Two month-old tissue-culture derived banana plantlets were used as experimental plants. Twenty plantlets were used as replicates for each of the techniques tested. Data from the infected plantlets were taken from 1 to 5 weeks after inoculation. Total percentage infection and disease severity index (DSI) based on the leaf symptom index (LSI) and rhizome discoloration index (RDI) were computed for each of the techniques used. Comparing the three techniques based on the computed DSI, results showed that the corn meal-sand inoculum technique gave the highest percent infection (90%). Highest LSI of 3.1 and highest RDI of 6.25 was also derived from plantlets inoculated using the corn meal-sand inoculum technique. The technique also showed lower statistical variation.

**Reporter Gene-Labelled *Mycosphaerella fijiensis* and *Fusarium oxysporum* f. sp. *cubense* as Tools for Pathogenicity Studies**

A.K. Kobayashi¹,² P.M. de Vries², C. Diaz-Trujillo², C. Waalwijk², T.A.J. van der Lee², L.V. Paiva⁴, M.T. Souza Jr²,³ and G.H.J. Kema²

¹Embrapa Mid-North, Av. Duque de Caxias, 5650, CEP 64006-220, Teresina/PI, Brazil; ²Plant Research International, PO Box 16, 6700 AA Wageningen, The Netherlands; ³Embrapa LABEX Europe, PO Box 16, 6700 AA Wageningen, The Netherlands; ⁴Universidade Federal de Lavras, Caixa Postal 3037, Lavras/MG, Brazil

Transgenic fungi expressing reporter genes have demonstrated to be useful tools for studies on pathogenicity in different pathosystems. Such methodology could also be suitable for studies on banana diseases. Among the different
diseases occurring in banana and plantain, black leaf streak caused by *Mycosphaerella fijiensis* and Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* (Foc) are the most destructive and important worldwide. We have used the *Agrobacterium*-mediated transformation system to develop strains of *M. fijiensis* and Foc expressing the reporter genes *gfp* (green fluorescent protein) and *dsred* (*Discosoma* sp. red fluorescent protein). Two gene constructs carrying either reporter genes driven by the constitutive promoter PtoxA were used. Both constructs also contain the *hph* gene for resistance to hygromycin as selective marker. We generated transgenic strains from *M. fijiensis* isolates of opposite mating types from different global populations, CIRAD86 (*mat1*-1, Cameroon) and CIRAD139A (*mat1*-2, Colombia), as well as of Foc tropical race 4 (VCG-01213) expressing either reporter gene separately. Plant-pathogen interaction studies using such reporter gene-labelled strains are currently in progress.

**Correlation between Susceptibility for Pathogen and Crude Toxin of *Fusarium oxysporum* f. sp. *cubense* race 4 in Banana Varieties Plantlets**

X.J. Yang, Y.X. Du, F.R. Chen, L. Gan and H.C. Ruan

*Institute of Plant Protection, Fujian Academy of Agricultural Sciences, Fuzhou, Fujian 350013, China*

A study was done to investigate the correlation between susceptibility for *Fusarium oxysporum* f. sp. *cubense* (Foc) race 4 and susceptibility for crude toxins of the pathogen in banana cultivars. The susceptibility of 36 banana cultivars to the pathogen was studied by inoculating plantlets’ roots with Foc race 4 conidial suspension. The susceptibility of five banana cultivars to crude toxins was studied by treating plantlets’ roots and leaves with crude toxins of Foc race 4. The results showed that four cultivars, namely ‘Tianbaojiaojiao’ (AAA), ‘Chaijiao’ (AAA), ‘Guangfen1’ (AAB) and ‘Gongjiao’ (AA) showed resistance to Foc race 4, and two cultivars, namely ‘Costarica’ (AAA) and ‘Kangku 5’ (AAA) showed intermediate resistance to Foc race 4. Foc race 4 was cultured in 100 ml Czapek in 150 cc conical flasks at 28ºC and 140 r/min for 10 days. The liquid medium was centrifuged at 5,000x g for 10 min and the supernatant toxic media prepared as tested crude toxins. The susceptible
cultivars showed higher susceptibility to the crude toxins than the resistant cultivars. Analysis of the difference of disease index, wilt index and leaves lesion area of 60 days-old plantlets and tissue-cultured plantlets showed that there is a positive correlation between the susceptibility for the pathogen and crude toxins of Foc 4 in banana cultivars, and a significant positive correlation between disease index and leaves lesion area of 60 days-old plantlets. Measuring the leaves lesion area in the middle leaves after treatment with crude toxins for 72 hours could thus be used as an effective method to identify the host reaction of banana cultivars to Foc race 4.

Field Evaluation of Banana Genotypes for Resistance to Fusarium Wilt

S.O. Silva¹, L.R. Ribeiro², E.P. Amorim¹, Z.C. Cordeiro¹, M.C. Lima² and M.A. Dita¹

¹Embrapa Cassava & Tropical Fruits, Cruz das Almas, 44380-000, Bahia, Brazil; ²Federal University of Reconcavo of Bahia, Cruz das Almas, 44380-000, Bahia, Brazil

Fusarium wilt of banana, caused by the soil-borne pathogen *Fusarium oxysporum* f. sp. *cubense* (Foc), is one of the most devastating diseases of this crop. No sustainable control strategy exists other than replacing susceptible cultivars with cultivars resistant to the disease. Identification of resistance sources is thus an essential step in breeding programmes. In Brazil, this is not only important for *Musa* genotypes used for consumption, but also for those used for ornamental purposes. In this work, 28 *Musa* genotypes with different ploidy levels were evaluated for resistance to Fusarium wilt, with the aim to: a) identify sources of resistance and b) characterise promising genotypes for disease resistance. Ten plants of each genotype were planted in an area highly infested by Foc races 1 and 2 using a completely randomised design. As susceptible control, ‘Silk’ (AAB) plants were placed between each individual. Disease severity was assessed monthly by means of a scale based on external symptoms ranging from 0 to 4 (0: healthy plant and 4: dead plant). In addition, harvested plants were evaluated for rhizome discoloration. Most of the ‘Silk’ plants (96%) showed symptoms of the disease, confirming the high inoculum pressure in the area. Highly resistant genotypes were identified in all ploidy levels. Most of the diploid genotypes were classified as resistant. Among them,
‘NBA-14’ (AA) was highlighted, since it produces commercial fruits with high carotenoids contents. Hybrids, such as ‘PA94-01’ (AAAB) and ‘FHIA-02’ (AAAA) were also ranked as resistant. These genotypes are promising for direct cultivar selection, introgression of resistance alleles in susceptible genotypes, as well as to elucidate the genetic basis of resistance to Fusarium wilt in banana.

**Reaction of *Musa* Hybrids to Fusarium wilt and Burrowing Nematode Complex**

T.N. Balamohan, Suken Chandra Das, K. Poornima and N. Seenivasan

*Department of Fruit Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India*

Root feeding injury by the burrowing nematode, *Radopholus similis*, will pave easy entry to *Fusarium oxysporum* f. sp. *cubense* (Foc) in banana. Hence, developing combined resistance to both nematode and Fusarium is considered a useful banana breeding target. Forty-three banana hybrids developed by crossing Fusarium- and nematode-resistant male parents, namely ‘Pisang Lilin’ (AA), ‘Anaikomban’ (AA), ‘Pisang Jari Buaya’ (AA), ‘Ambalakadali’ (AB), ‘Rose’ (AA), H-56, H-201 (BB) and ‘Yangambi Km5’ (AAA), with commercial triploid bananas ‘Karpooravalli’ (ABB), ‘Poovan’ (AAB), ‘Hill Banana’ (AAB), ‘Manoranjitham’ (AAA) and ‘Rasthali’ (AAB) were screened for their reaction to Foc (race 1) alone and in combination with *R. similis* in pots under glasshouse conditions. *Radopholus similis* (multiplied by carrot disc culture technique) and Foc (maintained in sand/maize medium containing 12 x 103 colony forming units/g) were inoculated in the rhizosphere of the plants at 5000 nematodes/pot and 10 g/pot, respectively, 15 days after planting. Resistance to *R. similis* was assessed by the root lesion index (%), with 0% – Immune, <10% – Resistant, 10-20% – Tolerant, 20-40 – Susceptible and >40% – Highly Susceptible). A wilt score was given based on the discoloration of the corm [scale 1-6, with 1 – Corm completely clean, no vascular discoloration (Resistant), 2 – Isolated points of discoloration in vascular tissue (Tolerant), 3 – Discoloration up to 1/3 of vascular tissue (Moderately susceptible), 4 – Discoloration between 1/3 and 2/3 of vascular tissues (Susceptible), 5 – Discoloration more than 2/3 of vascular tissue (Highly susceptible) and 6 –
Poster Presentations - Session 2

Total discoloration of vascular tissues (Highly susceptible). When the hybrids were inoculated with Foc alone, ‘H-511’, ‘H-516’, ‘H-531’, ‘H-534’, ‘H-537’, ‘H-571’, ‘H-02-34’, ‘H-03-05’, ‘H-03-13’, ‘H-03-17’, ‘H-04-12’ and ‘NPH-02-01’ were found resistant with a wilt score of 1.0. When Foc was inoculated along with R. similis, the hybrids ‘H-516’ and ‘H-531’ recorded a root lesion index of 7.0% and a wilt score of 1.0, and were thus rated as resistant to both fungus and nematode. The hybrids ‘H-511’, ‘H-534’, ‘H-537’, ‘H-571’, ‘H-02-34’, ‘H-03-05’, ‘H-03-13’, ‘H-03-17’, ‘H-04-12’ and ‘NPH-02-01’ were found to be resistant to Foc and tolerant to R. similis. Reduction of plant height, plant girth, number of leaves/plant and number of roots/plant after combined inoculation was lowest in ‘H-531’. Polyphenol oxidase, phenylalanine ammonia lyase enzyme activities and total phenol contents in roots were higher in ‘H-531’ than in the other hybrids. The screening trial indicated that the new banana hybrid ‘H-531’ shows good combined resistance to Foc and nematodes.

**Development of In-Vitro Rooted Banana Plantlets-Fusarium oxysporum f. sp. cubense Interaction System in Erlenmeyer Flasks**

Y.L. Wu, C.Y. Li, G.J. Yi, B.Z. Huang, Y.R. Wei, C.H. Hu and Y.H. Huang

_Fruit Tree Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou, 510640, P.R.China_

Fusarium wilt, caused by the soil-inhabiting fungus _Fusarium oxysporum_ f. sp. _cubense_ (Foc), has become the major constraint of banana production in China. Chemical control and existing cultural control measures such as flood fallowing, crop rotation and the use of organic amendments, have not been effective in managing the disease. Breeding for and selecting disease-resistant cultivars is generally accepted as the most sustainable method to maintain banana production. While field evaluation of genotypes remains the benchmark for evaluating host-plant resistance, it is cumbersome, time consuming and expensive. An in-vitro system to study banana rooted plantlets-Foc interaction has been developed, characterised by modified medium and in-vitro inoculation in Erlenmeyer flasks. The adjustment of medium components could not only guarantee the normal growth of plantlets, but also avoided plantlets being killed.
by profuse growth of Foc. The system proved easy to handle, and – being a totally closed system compared with pot system and hydroponic system – the culture conditions (especially temperature) could be better controlled than in the greenhouse. Since the activity of Foc is affected by environmental factors, the system established allows more consistent banana infection by Foc. For ‘Brazil Xiangjiao’ (AAA, Cavendish subgroup), rooted plantlets died within 20-25 days after the inoculation of race 4 of Foc, while they grew well after the inoculation of race 1. For cultivars with different levels of field resistance to race 4, in-vitro disease indexes of plantlets were much lower than that of the susceptible cultivar. In the Fruit Tree Research Institute, the system is now being used in rapid screening of Musa species for resistance to Fusarium wilt, study of induced resistance to Fusarium wilt and early events of banana infection by Foc.

Isolation and Characterisation of Endophytic Bacteria from Fusarium Wilt-Resistant Banana Plants and Evaluation of their Antipathogenic Activities against *Fusarium oxysporum* f. sp. *cubense*

R. Thangavelu, P. Ganga Devi, M. Gopi and R. Baby Shalini

*National Research Centre for Banana (NRCB-ICAR), Thogamalai Road, Thayanur (Post), Tiruchirapalli 620102, Tamil Nadu, India*

A total of 114 endophytic bacterial isolates were obtained from roots, corms, leaf lamina and mid-rib portions of 17 different banana accessions resistant to Fusarium wilt. All isolates were characterised by morphological, biochemical and molecular methods by sequencing 16S rRNA genes. These tests grouped the bacterial endophytes into nine different groups, namely *Actinomycetes, Serratia, Pseudomonas, Klebsiella, Azotobacter, Micrococci, Bacillus, Citrobacter* and *Staphylococcus*. The isolates were evaluated for their ability to inhibit spore germination and mycelial growth of *Fusarium oxysporum* f. sp. *cubense* (Foc) by cavity slide technique, dual culture plate and volatile production methods. Among the 114 endophytes screened, three isolates, namely *Actinomycetes* sp. 17Ra, *Klebsiella* sp. 17Rb obtained from the roots of ‘Yangambi Km5’ (AAA, Ibota subgroup) and *Staphylococcus* sp. 15Cb from a
corm portion of ‘Pisang Seribu’ (AAB), exhibited 90-100% inhibition of spore germination and 49-54% reduction of mycelial growth by volatile production and 60-74% reduction of mycelial growth by dual culture plate methods. Further, a study on the compatibility of these effective endophyte isolates with the fungicides Propiconazole and Carbendazim at different concentrations (1 to 0.01%) indicated that the three endophytes were compatible with up to 0.1% concentration of these two fungicides tested. The scope of using these effective endophytic bacterial isolates with the fungicides for the effective management of Fusarium bacterial wilt in banana is discussed.

**Acidifying Amendments and Fusarium Wilt Incidence in Banana in Indian Peninsula**

M. Edward Raja and P.N. Krishnamurthy

*Division of Soil Science and Agricultural Chemistry, Indian Institute of Horticultural Research, Bangalore, India*

Nitrogen use efficiency is generally low in Indian agriculture, due to monsoonic climate (heavy rainfall for 4 months alternating with drought), high pH of soils, broadcast method of application and semi-arid tropical climate. Hence, nitrification inhibitors, like neem seed cake (*Azadiracta indica*), are used as amendments in rice and banana cultivation in India. While known for its nematicidal properties, neem cake was found to enhance Fusarium wilt incidence in susceptible cultivars, like ‘Ney Poovan’ (AB). A study was initiated with three treatments: (T1) application of N at 250 g/plant as ammonium sulphate; (T2) application of N at 250 g/plant as calcium ammonium nitrate; and (T3) application N at 250 g/plant as ammonium sulphate and 1000 g neem cake. The experiment was conducted in a loamy alfisol with pH 6.8. All other nutrients and water were given as per recommendations. Evaluation of plants at 6 months after planting indicated that treatment T3 (ammonium sulphate + neem cake) resulted in 42% of the plants showing classical symptoms of Fusarium wilt (rhizome pitting, pseudostem splitting), compared with 22% for T1 (ammonium sulphate alone) and 12% for T2 (calcium ammonium nitrate) The pH of the rhizosphere soil was 6.5, 7.2 and 5.9 in T1, T2 and T3 respectively. The results indicate that ammonium sulphate and neem cake aggravate Fusarium wilt by rhizosphere acidification since N as
NH$_4^+$ ions (when nitrification inhibitors are used) encourages equivalent H$^+$ excretion by the roots to maintain ionic balance. This results in low pH known for its effect in enhancing availability of native iron favouring *Fusarium oxysporum* f. sp. *cubense*.

**Evaluation of host reaction of Musa germplasm to the Banana Corm Weevil, Cosmopolites sordidus**

B. Padmanaban, S. Uma and M.M. Mustaffa

*National Research Centre for Banana (NRCB-ICAR), Thogamalai Road, Thayanur (Post), Tiruchirapalli 620102, Tamil Nadu, India*

‘Pisangrajabulu’, ‘Poovan’ (0197 and 0294), ‘Rasthali’ (0297), ‘Saapkal’ and ‘Terabun’. None of the accessions was resistant, except *Musa laterita* of the Rhodochlamys section. The present investigation of corm weevil screening resulted in the identification of 51 moderately resistant sources, which could be further used for breeding.

### Evaluation of Seeded Banana for Biotic Stresses

M.A. Hasan¹, R. Ray Chowdhury¹, M. Manna¹, K.K. Mandal¹, D. Majumder² and S. Jha³

¹Department of Fruits and Orchard Management, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India; ²Department of Agricultural Statistics, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India; ³Department of Agricultural Entomology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Thirty-four (34) seeded banana types (*Musa* BBB/BB) were evaluated for biotic stresses, i.e. Bunchy top, Sigatoka, Fusarium wilt, leaf-scarring beetle and pseudostem weevil under natural infection at the Horticultural Research Station, West Bengal, India during the years 2005-2008. The intensity of infestation was scored by coding 0 = inapplicable, 1 = very low or no visible sign of susceptibility (CR: completely resistant), 3 = low (HR: highly resistant), 5 = intermediate (MR: moderately resistant) and 7 = high (S: susceptible). Different multivariate techniques were used for analysis, namely proximity matrix, single linkage hierarchical clustering of square Euclidean distance matrix, principal component analysis (PCA) and multidimensional scaling. Clustering analysis grouped ‘Seed Banana-4’ (BBB) and ‘Seed Banana-6’ (BBB) in one cluster, while ‘Kalyani Local-4’ (BBB) and ‘Seed Banana-13’ (BBB) formed another cluster. In PCA, two factors were considered having Eigenvalues more than 1, explaining 58.70% of the total variation in the factorial distribution of seeded banana types. ‘Kalyani Local-4’, ‘Seed Banana-13’, ‘Mondouri Local-2’ (BBB), ‘Kalyani Local-2’ (BBB) and ‘Seed Banana-6’ (BBB) were most susceptible to Sigatoka and bunchy top, while ‘Seed Banana-7’ (BBB), ‘Maricha’ (BBB), ‘Seed Banana-15’ (BBB), ‘Jhama Daira’ (BBB), ‘Seed Banana-18’ (BBB) and ‘Baruipur’ (BB) were more prone to leaf-scarring beetle and Fusarium wilt. The scatter diagram between Factor-1 vs. Factor-2.
clearly indicated that the accessions ranked in the order of ‘Kalyani Local-4’ > ‘Seed Banana-13’ > ‘Mondouri Local-2’ > ‘Kalyani Local-2’ in terms of susceptibility to bunchy top and Sigatoka. The accessions ranked in the order of ‘Seed Banana-6’ > ‘Seed Banana-4’ > ‘Maricha’ > ‘Hill Banana’ in terms of weevil and Fusarium wilt susceptibility.

Screening of Banana Germplasm for Resistance/Tolerance to *Pratylenchus coffeae*

P. Sundararaju, T. Sekar, S. Uma, P. Saravanan and M.M. Mustaffa

*National Research Centre for Banana (NRCB-ICAR), Thogamalai Road, Thayanur (Post), Tiruchirapalli 620102, Tamil Nadu, India*

The root-lesion nematode, *Pratylenchus coffeae*, is an important banana nematode, causing considerable yield loss. It is present in almost all banana-growing countries. Host-plant resistance is advocated as one of the most sustainable nematode management practices. Banana germplasm available at the field genebank of NRCB Farm, Tiruchirapalli, India was evaluated against *P. coffeae* under field conditions. Accessions belonging to different genomic groups, i.e. AA, AB, AAA, AAB and ABB, were analysed based on various plant growth parameters and host response in terms of root-lesion indices and nematode populations from soil and roots. Field observation indicated that some of the accessions were free from root-lesion nematode infestation. Twelve diploids (AB) and 48 triploids (AAB) were further screened in pots under greenhouse conditions against *P. coffeae* during 2007-08. The diploids ‘Kunnan’, ‘Gragric Sarpara’ and ‘Narmin’ and the triploids ‘Dasaman’, ‘Kottavazhai’ and ‘Sakkar Chyana’ were found resistant to *P. coffeae*, whereas the diploid ‘Valiya Kunnan’ and the triploids ‘Ladies Finger’ and ‘Cheni Champa’ were found moderately resistant to the root-lesion nematode. The triploids ‘Sirumalai’, ‘Garomoina’, ‘Malavazhai’ and ‘Pacha’ recorded high root-lesion indices and nematode populations, but showed minimal effect of the nematodes on the plant growth, which indicates that they posses a high degree of tolerance to *P. coffeae*. The remaining 47 accessions, both diploids and triploids, were found susceptible/highly susceptible to *P. coffeae*. 
Banana Nematode Control using Argan and Other Medicinal Plants

Z. Ferjī¹, H. Mayad², L. Bouhaddou¹ and D. De Waele³

¹Laboratoire de Nématologie, Institut Agronomique et vétérinaire Hassan II Complexe Horticole d'Agadir; B.P 18/S, Agadir. Marocco; ²Laboratoire de Symbiotes Racinaires et Biochimie végétale, Faculté des Sciences, B.P. 28/S, Agadir, Marocco; ³Laboratory of Tropical crop Improvement, Catholic University of Leuven (KUL), K. Mercierlaan 92, 3001 Heverlee, Belgium

A pot experiment was carried out at the Institut Agronomique et Vétérinaire Hassan II in Agadir, south of Morocco in order to evaluate the efficiency of some organic amendments on the control of *Meloidogyne* spp. and *Helicotylenchus multicinctus* in ‘Giant Cavendish (AAA). One-month old in-vitro banana plants were planted in a field naturally infested with *Meloidogyne* spp. and *H. multicinctus*. Treatments included: (1) grounded castor leaves at 50 and 100 g, (2) grounded castor seeds at 50 and 100 g, (3) a cake from the indigenous Argan tree (*Argania spinosa*) at 150 g and (4) a control (not treated). The treatment efficiency was evaluated by measuring the *Meloidogyne* and *H. multicinctus* population densities in the soil as well as the root gall index (RGI). A decrease in nematode population was observed for all treatments in comparison with untreated plants. Argan cake allowed the highest nematode reduction (up to 100%). Also, only a few small galls were formed on Argan cake-treated plants (RGI = 0,42) compared with the control (RGI = 4,42). An improvement of plant growth was also noticed on treated plants. The best root weight (27,72 g), aerial plant parts weight (225,31 g), plant height (64,8 cm) and stem circumference (10,87 cm) were obtained for Argan-treated banana plants. The results indicate that the cake of the Moroccan indigenous Argan tree can be used as an efficient and environmentally safe control method against *Meloidogyne* spp. and *H. multicinctus* associated with banana.
Screening of Banana Varieties/Germplasm against Foliar Diseases

A.N. Sabalpara, Priya John, K.U. Solanky and B.P. Mehta

Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, Navsari 396 450, Gujarat, India

Banana is one of the major fruit crops grown in south Gujarat (India). Sigatoka leaf spot caused by *Mycosphaerella musicola* is one of the major constraints to profitable cultivation of banana. Sixty-seven cultivars were evaluated under natural conditions for 9 years in the same field. The plants were planted in a completely randomised block design in a plot size of 7.2 x 7.2 m with a spacing of 1.8 x 1.8 m with three replications. Each plot accommodated 16 plants. The centre four (ring) plants were tagged for observations. To provide maximum inoculum, a susceptible variety was planted around the test cultivars. Crop debris was also added every year. The sampling and identification of the pathogen was carried out as per the INIBAP Technical guidelines, and the pathogen was identified as *M. musicola*. The recommended agronomic practices were practiced. Fertiliser was applied at 200-90-200 g NPK/plant in four splits. Disease development was measured from the stage of unrolling of the banana leaf to the development of 10 mature necrotic lesions stage with inspection of plants once a week. The infection index was measured as per Gauhl’s modification of Stover’s disease severity and grading as per the method followed by All India Coordinated Research Project on banana (0-5 – Resistant; 5-15 – Moderately resistant; 15-25 – Moderately resistant; >25 – Susceptible). Eight varieties were found resistant, i.e. ‘Then Kunnan’ (AA), ‘Karim Kadali’ (AAA), ‘Terabun’ (genome not known), ‘Annai Komban’ (AA), ‘Nikhanka’ (genome not known), ‘Pocha Kunnan’ (ABB), ‘Mutheli’ (AAB) and ‘Champa Dhati’ (genome not known). Nineteen varieties were moderately resistant, whereas nine varieties were found moderately susceptible. The remaining 31 varieties were found susceptible to leaf spot.
The Importance of Black Leaf Streak Control in ‘Pisang Berangan’ (AAA) in Peninsular Malaysia

K.H. Then and S. Palaniappan

_Felda Agricultural Services Sdn. Bhd., Malaysia_

Black leaf streak, caused by _Mycosphaerella fijiensis_, is the most destructive leaf disease of ‘Pisang Berangan’ (AAA) in Malaysia. It leads to significant decline in yield and fruit quality due to reduction of photosynthetic sites in the functional leaves. Several fungicides, such as a.i. benomyl, tridemorph, mancozeb and propiconazole, were evaluated for their potential to control the disease in ‘Pisang Berangan’. The study showed that both propiconazole and benomyl are effective in reducing the disease, with treated plants having an average of 15.6 functional leaves as compared to 8.0 functional leaves in untreated plants at 4 months after treatment. Spraying of propiconazole alone or alternatively with benomyl at monthly intervals resulted in an increase in bunch weight to 21.31 kg and 21.29 kg, respectively, as compared with 15.67 kg for untreated plants. Spraying of benomyl alone also improved the bunch weight to 18.36 kg. Generally, the effective black leaf streak control by the above fungicides resulted in yield improvement of about 17.2%-36.0% and better income of up to 23.6%-46.6%. Therefore, the implementation of black leaf streak control is important and necessary for ‘Pisang Berangan’ cultivation in Malaysia, especially in the first generation of the crop, to ensure better profitability.

Isolation and Characterisation of Endophytic and Epiphytic Bacteria from Sigatoka Leaf Spot-Resistant Plants and Evaluation for their Antipathogenic Activities against _Mycosphaerella_ spp.

R. Thangavelu, R. Baby Shalini, M. Gopi and P. Ganga Devi

_National Research Centre for Banana (NRCB-ICAR), Thogamalai Road, Thayanur (Post), Tiruchirapalli 620102, Tamil Nadu, India_

A total of 48 endophytic and 34 epiphytic bacterial isolates were obtained from the mid rib and leaf lamina of 18 different banana accessions resistant to
Sigatoka leaf spot diseases caused by *Mycosphaerella* spp. All these isolates were characterised by morphological, biochemical and molecular methods by sequencing 16S rRNA genes. These tests grouped the bacterial endophytes into five major groups, namely *Serratia*, *Klebsiella*, *Actinomycetes*, *Citrobacter* and *Staphyloccocus*, and the epiphytic bacterial isolates into two major groups, namely *Pseudomonas* and *Bacillus* spp. All the endophytic and epiphytic isolates were evaluated for their in-vitro antipathogenic activities by different methods, such as cavity slide technique, filter paper disc and volatile production. The result of these studies show that among the 48 endophytes screened, the isolates *Serratia* sp. 6M2, *Klebsiella* sp. 2M and *Actinomycetes* 14M2 isolated from the mid rib of ‘Kalibow’ (AAB), ‘Cv. Rose’ (AA) and ‘Pisang Rajah’ (AAB), respectively, exhibited more than 95% inhibition of spore germination by cavity slide technique, 100% inhibition of mycelial growth by volatile production and more than 4 cm of inhibition zone by filter paper disc method. Of the 34 bacterial epiphytes, *Pseudomonas* sp. isolates 1E2 obtained from ‘Calcutta-4’ (AA) and 5E2 from ‘GCTCV-119’ (AAA) and *Staphyloccocus* sp. 6E from ‘Kalibow’ (AAB) exhibited 90-94% inhibition of spore germination by cavity slide technique, 92-100% inhibition of mycelial growth by volatile production and 3.9-4.3 cm of inhibition zone by filter paper disc method. Compatibility of these effective endophytes and epiphytes with certain fungicides effective against Sigatoka disease, such as propiconazole, tridemorph, carbendazim and mancozeb at different concentrations (1 to 0.01%), was carried out under in-vitro conditions. The results show that most of the endophytes and epiphytes are compatible with up to 0.1% concentration of all the fungicides. The scope of using these effective endo- and epiphytic bacterial isolates with the fungicides for the effective management of the Sigatoka disease is discussed.

**Community Coping Mechanisms in Response to Xanthomonas Wilt Epidemics in Uganda**

E. Karamura¹, L. Aliguma² and W. Tinzaara¹

¹Bioversity International, PO Box 24384, Kampala, Uganda; ²Ssemwanga centre, Kampala, Uganda

The study was conducted in Mukono district, Central Uganda, one of the major banana-growing regions with the longest history of Xanthomonas wilt
incidence. The district grows mainly beer and dessert bananas though green cooking East African highland bananas are also important. Since 2001, the region was heavily infected by Xanthomonas wilt with crop losses up to 90%. The disease subsequently spread to other banana-growing regions of the country. It was however not clearly understood how the banana-dependent communities were coping with such high crop losses. The study was conducted in 2005, to identify and document the coping strategies employed by various actors along the production-consumption chain in the face of the devastating disease. The study also considered the viability of the strategies employed in terms of affordability, availability, sustainability and effectiveness, as a basis for increasing knowledge about the effects of the disease. The study revealed that actors along the production-consumption chain have adopted various strategies to cope with the disease. Additionally, the majority of the farmers were growing other crops, such as maize and beans, to raise income, and traditional banana traders had diverted their capital to other crop enterprises. Animal production, especially dairy cattle, attracted most affected farmers (6%) and grain produce trade the least (3%). Post-harvest processors were forced to reduce the quantities being processed, while others started processing alcoholic drinks from other crops. A few others (on average 8%) abandoned banana business completely. Between 2001 and 2005, the volume of matooke and banana leaves sold in the local market went down by 75% and 50%, respectively. For the same period, the respective price increases for both matooke and banana leaves were 70% and 20%. The effects of the increase in prices however did not translate into increased incomes at the household level because the volumes sold were reducing with increased pressure from the disease. The overall picture was one of reduced incomes, and the quality of life in most households has deteriorated between 2001 and 2005 due to the Xanthomonas wilt outbreak.

The Drivers of Banana Xanthomonas Wilt Epidemic in East and Central Africa

W. Tinzaara and E.B. Karamura

*Bioversity International, PO Box 24384, Kampala, Uganda*

Xanthomonas wilt of banana, caused by the bacterium *Xanthomonas campestris pv. musacearum*, is one of the major threats to banana production in
East and Central Africa. The disease was initially identified in Ethiopia in 1960 on *Ensete ventricosum* (a close relative of banana) and it emerged in Uganda in 2001. Xanthomonas wilt has now been confirmed in all the countries of the Great Lakes region, except Burundi which stands threatened. The disease is indiscriminately attacking all banana cultivars and results in total yield loss once it establishes itself. One of the major challenges to the management of the disease has been poor understanding of the factors influencing disease spread and severity. This study aimed at determining the role of host plant-vector interaction and cultural practices in influencing Xanthomonas wilt distribution and incidence. Data were collected using survey studies focusing on distribution and incidence with regard to cultivar and management. The results indicate that vectors are the major drivers of disease distribution and incidence among the ABB banana cultivars. Cultural practices together with movement of banana planting material are the key drivers of disease distribution and incidence among the AAA banana cultivars. The results of this study will be of assistance in developing and targeting of management practices for Xanthomonas wilt.

**Bacterial Rhizome Rot – A New Threat to French Plantain Cultivar ‘Nendran’ (*Musa*, AAB) in India**

A. Cherian¹, N.K. Usha² and R. Menon¹

¹Banana Research Station, Kerala Agricultural University, Kannara PO, Thrissur, Kerala, India; ²Department of Plant Pathology, College of Horticulture, Vellanikkara PO Thrissur, Kerala, India

The French plantain cultivar ‘Nendran’ (*Musa*, AAB) is one of the most widely grown cultivars of banana in South India. Especially in the state Kerala, which has a humid tropical climate, banana cultivation has been extended to fields previously used for paddy. The humid microclimate prevalent in these areas has led to a new disease, named bacterial rhizome rot. Surveys conducted in different parts of the state revealed that the occurrence of the disease is more frequent from May to August, when there is heavy rainfall and high humidity. Etiological investigation confirmed that the disease is caused by the bacterium *Erwinia carotovora*. The key diagnostic symptoms are flaccidity and yellowing of leaves followed by toppling down of the plants. The rhizome shows severe
rotting with foul smell. In the field genebank, the disease was also recorded in others cultivars, like ‘Rasthali’ (AAB), ‘Robusta’ (AAA) and ‘Populu’ (AAB). Pot culture studies were conducted to evolve recommendations to manage the disease. Untreated control plants were also included. The results showed that the disease could be effectively managed by drenching soil around the infected plants with copper oxychloride at 0.4% and streptocycline at 300 ppm. The biocontrol agent, *Pseudomonas fluorescens*, botanical garlic extract and bleaching powder were also found to be effective and could be used in the integrated management of the disease.

**Current Status, Diagnosis and Management of Viral Diseases of Banana in Kerala, South India**

A. Cherian, R. Menon, P.M. Mathew, A. Suma and K.C. Aipe

*Banana Research Station, Kerala Agricultural University, Kannara PO, Thrissur, Kerala, India*

Kerala, the southern most state of the Indian peninsula, enjoys a humid tropical climate. The French plantain ‘Nendran’ (AAB) is the preferred cultivar in this state and occupies the first place among the commercially cultivated varieties in Kerala. Other varieties include ‘Robusta’ (AAB), ‘Rasthali’ (AAB), ‘Mysore Poovan’ (AAB) and others. Surveys conducted throughout the state recorded only four viral diseases, namely banana bunchy top, bract mosaic, infectious chlorosis and streak disease. The extent of yield loss due to these viruses ranged from 50-100%. The etiology was confirmed by electron microscopy and sap transmission. The field genebank maintained at the Banana Research Station, Kannara is screened regularly for identifying sources of resistance. Wide variation in symptomatology was recorded in different clones of the genebank. The serological and molecular diagnosis of these viruses was standardised and is routinely used for indexing tissue culture as well as sucker planting materials which are distributed to the farmers of this state. Strategies developed for the integrated management of these virus diseases will also be discussed.
Symptomless Banana Plants and Other Species as Reservoir of BBTV Inoculum

F. dela Cueva¹, E. Dinglasan¹, F.S. dela Cruz¹, V. Sinohin² and A.B. Molina²

¹National Plant Genetic Resources Laboratory, Crop Science Cluster, Institute of Plant Breeding, University of the Philippines Los Baños, College, Laguna 4031, Philippines;
²Commodities for Livelihoods programme, Bioversity International - Asia Pacific, 3F Khush Hall, IRRI, Los Baños, Laguna, Philippines

The use of healthy planting materials derived from tissue culture is the focal strategy in the management of bunchy top disease, caused by the *Banana bunchy top virus* (BBTV), in the Philippines. This is coupled with a community approach of eradication of infected plants in order to reduce sources of inoculum. This study was carried out to determine whether plants without BBTV symptoms can act as possible reservoirs of inoculum. Suckers from asymptomatic plants were collected from ‘Lakatan’ (AAA) and ‘Bungolan’ (AAA), both BBTV-susceptible dessert cultivars, and from ‘Saba’ (BBB), a popular cooking cultivar resistant to BBTV. Plant samples were collected from communities where bunchy top is a recognised problem. Presence or absence of the BBTV virus in the sample plants was determined using enzyme-linked immunosorbent assay (ELISA) and aphid transmission tests. Results of ELISA showed that most of the symptomless plants were infected with the virus. Transmission tests from positive samples to tissue culture-derived plants of ‘Lakatan’ confirmed positive infection. Typical symptoms of the disease such as vein clearing and marginal chlorosis started to appear 21 days after inoculation feeding. Severe stunting and necrosis developed 2 months after inoculation. Some common weeds and crop species were also subjected to insect transmission and ELISA tests. Presence of BBTV virus was detected by ELISA and positive transmissions were obtained from *Heliconia* sp., *Canna* sp. and *Colocasia* sp. (both common and ornamental species). Symptoms started to appear 4 weeks post insect transmission on variegated taro, *Colocasia bicolor*, and *Heliconia* sp. as marginal chlorosis and mosaic, respectively. The results showed that asymptomatic banana plants within an area with BBTD, regardless of cultivar, can serve as infection foci for the spread of BBTD. Other non-Musacea crop species identified in this study can also serve as alternate hosts of the virus and thereby serve as sources of inocula.
CR-M: An Important Nucleotide Acid Sequence to Distinguish BBTV Isolates from Two Groups

Y.H. Huang, J.W. Zeng, Y.L, Wu, R. Xia and G.J. Yi

Fruit Tree Research Institute, Guangdong Academy of Agricultural Science, Guangzhou, China

A new full nucleotide sequence of BBTV 6 was cloned and analysed. The results showed it contained two main characteristic sequences: the CR-SL (Stem-loop common regions) and the CR-M (Major common regions). The phylogenetic tree, using amino acid sequences of BBTV 6, suggested that BBTV contained two groups. The nucleotide and amino acid sequences of six BBTV components were aligned and compared. The highest identities of nucleotide and amino acid sequences within each component were 96.10% and 98.27%, and between the six components 70.78% and 56.24%, respectively. All the nucleotides sequences of the six BBTV components contained a major open reading frame (ORF), except BBTV 2. All six components also incorporated characteristic sequences such as CR-SL, CR-M, potential TATA box and potential polyadenylation signal. However, these characteristic sequences were somewhat different in different components. Each CR-M of all six BBTV components contained a 16-nucleotide GC-rich region. Furthermore, an almost complete 16-nucleotide direct repeat (ATACAAc/ gACa/gCTATGA) was located from 4nt to 19nt and from 21nt to 36nt in isolates from the South Pacific group, but not in isolations from the Asian group. The phylogenetic trees using full nucleotide sequences and amino acid sequences of six BBTV components indicated six groups corresponding to each BBTV component. The CR-M nucleotide sequence indicated two groups according to the place of origin. In addition, the secondary structure and some physicochemical properties of proteins of the six BBTV components were predicted, showing they had significant differences.
Towards Transgenic Resistance to *Banana Bunchy Top Virus (BBTV)* by Expression of Defective Viral Reps

A.M. Njoroge\(^1\), R.J. Geijskes\(^1\), R.M. Harding\(^1\), A.P. James\(^1\), T.T. Tsao\(^1\), D.K. Becker\(^1\) and J.L. Dale\(^1\)

\(^1\)Centre for Tropical Crops and Biocommodities, Queensland University of Technology, Brisbane, 4001, Queensland, Australia; \(^2\)Kenya Agricultural Research Institute, Thika, Kenya

Banana (*Musa* spp.) is one of the most important food crops in the world and provides a staple food and source of income for many households, especially in Africa. Diseases are a major constraint to production, with banana bunchy top generally regarded the most important viral disease affecting banana. Bunchy top disease is caused by the *Banana bunchy top virus* (BBTV), the type member of the genus *Babuvirus* in the family *Nanoviridae*. The integral genome of BBTV comprises at least six circular, single-stranded (css) DNA components known as BBTV DNA-R, -U3, -S, -M, -C and -N. BBTV DNA-R encodes the master replication initiation protein (Rep), which is the only viral protein essential for BBTV replication. Although bunchy top disease has been managed in Australia using strict phytosanitary measures, there are no effective control strategies in other countries where the disease continues to cause significant yield losses. The lack of any known BBTV-resistant cultivars has precluded the use of conventional breeding as a disease control strategy and, as such, genetically engineered resistance is considered the most promising approach to generate BBTV resistance in banana. One of the most promising molecular strategies used to generate resistance to other ssDNA viruses has been the use of trans-dominant negative defective Reps which interfere with virus replication. We are using a similar strategy in an attempt to generate transgenic resistance to BBTV by transforming banana embryogenic cell suspensions with three different defective BBTV Rep constructs. Progress towards obtaining BBTV resistance utilising this strategy will be reported.
Banana Tissue Culture for Production of *Banana Bunchy Top Virus* (BBTV)-Free Plants in Pakistan

A. Muhammad¹, H. Rashid¹, I. Hussain¹, S. Masood¹ and S.M. Saqlan Naqvi²

¹Agriculture Biotechnology Programme, National Agriculture Research Centre, Park Road, 45500, Islamabad, Pakistan; ²Department of Biochemistry, University of Arid Agriculture Rawalpindi, Pakistan

In Pakistan, banana is cultivated over an area of 32,500 hectares with an annual production of 163,500 tonnes. Dwarf Cavendish’ (‘Basrai’, AAA, Cavendish subgroup) occupies 98% of the area under banana cultivation. *Banana bunchy top virus* (BBTV) infection has decreased banana production by upto 60% in the country. The virus is spreading to new fields via infected suckers and through its vector, the black aphid (*Pentalonia nigronervosa*). Production of virus-free “Basrai” plants through tissue culture was studied. In-vitro cultures were established from field-grown suckers. Before in-vitro multiplication, the plantlets were BBTV indexed by polymerase chain reaction (PCR). During in-vitro multiplication, benzylaminopurine (BAP) significantly affected the shoot multiplication. The maximum shoot number (5.44/explant) was achieved on liquid medium having 4.0 mg/L BAP and 1.0 mg/L indole acetic acid (IAA). When kinetin was used instead of BAP keeping all other parameters same, the maximum shoot number was 4.33/explant. Multiplication rate was 1.8, 2.74, 3.42, 3.38 and 2.84 after 1ˢᵗ, 2ⁿᵈ, 3ʳᵈ, 4ᵗʰ and 5ᵗʰ subculture, respectively, indicating that multiplication rate declined after the 3rd subculture. Shoot tips which initially had a higher rate of multiplication continued this behaviour in succeeding subcultures. In-vitro rooting of banana shoots was achieved on MS medium. Use of IAA or indole butyric acid (1.0 mg/L) resulted in an increase in the root number and length to 8.6 and 6.8 cm, respectively. Agronomic traits of tissue culture-raised plants were compared with conventionally propagated plants in the field. Results showed that tissue-culture plants were taller than suckers (8.1%), produced more suckers (31%), flowered earlier (2.8%) and produced more fingers per bunch (9%).
Establishment of Banana Virus-Indexing Centres and Surveying for Banana Viruses in East Africa

A.P. James¹, J. Mugini², C. Changa³, J. Kubiriba³, L. Karanja⁴, R.J. Geijskes¹, R.M. Harding¹ and J.L. Dale¹

¹Centre for Tropical Crops and Biocommodities, Queensland University of Technology, Brisbane, Queensland, 4001, Australia; ²Mikocheni Agricultural Research Institute, Dar Es Salaam, Tanzania; ³National Agricultural Research Organisation, Kampala, Uganda; ⁴Kenya Agricultural Research Institute, Njoro, Kenya

Six viruses are currently known to infect banana worldwide. Of these viruses, *Banana bract mosaic virus* (BBrMV) and *banana virus X* (BVX) have not been detected in East Africa, but could be devastating if introduced. *Banana bunchy top virus* (BBTV) has been reported widely in central and southern Africa but has not been recorded in Kenya, Uganda and Tanzania. This virus poses the greatest risk to banana production in these countries, and continued exclusion is paramount. *Banana streak viruses* (BSV) are widespread in Uganda and probably other East African countries. Estimates on yield losses caused by streak disease vary, and characterisation of viruses causing this disease continues to be a priority in East Africa. *Cucumber mosaic virus* (CMV) may sporadically infect banana and cause a decrease in production. Diagnostic testing facilities are integral for early detection of new disease incursions. With the increasing use of tissue-cultured (TC) banana for field plantings in East Africa, virus indexing of source plants has become a priority to prevent the dissemination of virus-infected plants. Control strategies must be implemented across East Africa to minimise the potential impact of these viruses on banana production. At present, restrictions on germplasm movement and dissemination, and quarantine processes to discourage long-distance movement without quality assurance is lacking. With the development of capacity in virus indexing within the region, these quality assurance issues can be addressed, allowing virus-indexed germplasm to be exchanged and made available to TC nurseries for commercial supply. In addition to the establishment of centres for provision of virus indexing services and indexed plant material, surveys are being undertaken to establish the occurrence and distribution of known banana viruses. This paper will present a report on the present status of banana virus-
indexing centres in East Africa as well as an analysis of surveys undertaken in 2008-2009 in Uganda, Malawi, Tanzania and Kenya.

**Novel Approaches for Identifying Nematode Problems in Tissue-Culture Banana in India**

P. Sundararaju and M.M. Mustaffa

_National Research Centre for Banana (NRCB-ICAR), Thogamalai Road, Thayanur (Post), Tiruchirapalli 620102, Tamil Nadu, India_

Plant-parasitic nematodes are considered a serious pest on banana and plantain, causing severe root necrosis and galling, especially in tissue-culture plants. The root-knot nematode, *Meloidogyne incognita*, produces characteristic galling on the roots, thereby blocking the conducting vessels for the transport of major and minor nutrients. This nematode is predominantly observed in tissue-culture plants, even at the primary and secondary hardening stages. Heavily infested seedlings fail to establish in the field as the newly formed roots get infected. A preliminary survey carried out with various banana tissue-culture companies has indicated heavy root-knot nematode infestation at secondary hardening stages as the companies seldom use sterilised soil mixture. The nematode-infested plants exhibited retardation of plant growth with marginal yellowing of leaves. Such plants produced heavy galling on the roots, and the entire roots were damaged due to the nematode penetration and multiplication. Nematode larvae, eggs and egg masses were also seen from the cross section of the plant roots. It was observed that higher nematode infestation was recorded in tissue-culture plants than in conventional planting materials. Root samples collected from field plants also exhibited heavy root galling and necrosis. The percentage of root-knot nematode infestation ranged from 25 to 45% during a survey of a tissue-culture banana field in Tamil Nadu. Maximum nematode infestation was recorded in ‘Robusta’ (AAA) and ‘Grand Naine’ (AAA) (52%), followed by ‘Ney Poovan’ (AB) (46%), ‘Poovan’ (AAB) (42%) and ‘Nendran’ (AAB) (40%). Strategies of integrated nematode management to be adopted in tissue-culture companies as well as in the field are discussed in this paper.
Investigation into Low-Cost Medium for Hardening of In-Vitro Banana Plantlets

B. Jhurree-Dussoruth and H. Kallydin

_Agricultural Research and Extension Unit, Q. Bornes, Republic of Mauritius_

With the drop in both the price and quota of sugar for world export, the sugar industry in Mauritius has collapsed. In response, many sugarcane growers have opted for large-scale plantation of banana. This has resulted in an increase in the demand of quality and clean planting material of ‘Petite Naine’ (AAA), the main cultivar because of its short stature, good yield and resistance to _Fusarium oxysporum_ sp. _cubense_ race 1. This pathogen is partly responsible for wiping out a famous local ladyfinger-like banana. As suckers from the existing old plantations represent high risk of _Cosmopolites sordidus_ and viral disease transmission, the demand for disease-free, tissue-culture banana plantlets is gaining importance. However, the price of those plantlets from commercial laboratories is too high (about 3-4 times more than suckers), due to –among others – the reliance on imported potting mixes (peat, vermiculite) for the weaning of plantlets. With the objective of finding low-cost potting mixes for weaning, a trial was set up using soil and manure mix, locally available by-products from the sugar industry (scum, flyash and bagasse), cocopeat (after use from soil-less cultures) and imported jiffy pellets. After deflasking, plantlets (2.5 cm tall) of ‘Petite Naine’ were placed in eleven potting mixes [Scum, Flyash, Flyash + Scum (2:3), Bagasse + Flyash (1:1), Flyash + Scum (1:1), Flyash + Scum (3:2), Flyash + Scum (7:3), cocopeat, jiffy pellets, non-sterile soil + cow manure (1:1) and autoclaved soil + cow manure (1:1)]. Plantlets were placed in a shed (70% shade) with a simple structure made of transparent plastic placed over the trays to maintain humidity for 10 days. The number of dead plantlets, plantlet height and leaf emission were recorded. Mortality was negligible, indicating that the non-sterile mixes did not cause any disease incidence. Plantlet development was more rapid in sterile soil + manure mix and least in cocopeat and flyash/bagasse mixture. Low-cost options for the weaning step, which could reduce hardening costs by over 90%, were thus developed.
Performance of Banana Cultivars (*Musa* spp.) Propagated by Tissue Culture and Suckers in India

M.H. Dahale

*College of Horticulture, Dr PDKV Akola 444104, India*

A field experiment on the performance of different banana cultivars propagated by tissue culture (TC) and suckers (S) was undertaken at Marathwada Agricultural University, Parbhani, India. The experiment was laid out in randomised block design with seven Cavendish (AAA) cultivars and four replications. Four exotic cultivars (‘Williams’, ‘China Cavendish’, ‘Grand Nain’ and ‘Valery’), were propagated by TC, while one indigenous cultivar was propagated by TC and S (‘Basrai’) and two indigenous cultivars by S only (‘Ardhapuri’ and ‘Shrimanti’). A total of 25 S and TC plants were planted in each bed, out of which nine plants were selected randomly for observation. Amongst the exotic and indigenous cultivars studied, ‘Williams’ propagated by tissue culture recorded the best overall performance. Detailed results are presented in the paper.

Micropropagation of 'Pisang Awak' (*Musa*, ABB genome) as a Model for Enhancing and Improving Livelihood of Rural Communities in Malaysia

L.K. Chan¹, V.H. Au¹, A.A.A. Noor², M. Adnan³ and P.L. Boey⁴

¹School of Biological Sciences, Universiti Sains Malaysia (USM), 11800 Penang, Malaysia; ²School of Industrial Technology, USM 11800 Penang, Malaysia; ³School of Arts, USM, 11800 Penang, Malaysia; ⁴School of Chemical Sciences, USM, 11800 Penang, Malaysia

In Malaysia, banana is the second most commonly grown fruit crop, and approximately 50% of the banana-growing land in Malaysia is cultivated with ‘Pisang Berangan’ (AAA genome), ‘Pisang Rastali’ (AAB genome) and ‘Pisang Mas’ (AA genome). ‘Pisang Awak’ (ABB genome) is cultivated only by small holders for local consumption as dessert fruit. The recent discovery that unripe fruits of ‘Pisang Awak’ can be processed into high-fibre flour that
can be used to substitute wheat flour has encouraged many local farmers to plant this local cultivar commercially. However, large-scale development is limited due to insufficient supply of good-quality planting materials. Propagation of ‘Pisang Awak’ by suckers and by conventional tissue-culture using gel-medium was found to be slow and labour intensive. Our research team has successfully established an efficient micropropagation protocol that can be used for ‘Pisang Rastali’, ‘Pisang Berangan’ and ‘Pisang Awak’. Solid Murashige and Skoog (MS) medium was used throughout with shoot explants first cultured on medium supplemented with 5 mg/L BA for shoot multiplication. Rooting was best initiated on MS medium without growth regulators. The rooted plantlets were acclimatised by transplanting them into organic soil : top soil : sand mixture (1:1:1) for 2 weeks. This technology can be transferred to village communities throughout Malaysia, involving the setting up of a tissue-culture laboratory together with flour- and paper-producing facilities. As such, the farmers will have access to tissue-cultured plantlets for large-scale planting of ‘Pisang Awak’. The unripe fruits can be used to produce high-fibre flours while the banana tree trunks can be converted into papers. This model is being tested in a rural village, Kampong Perlis, at Balik Pulau, Penang, Malaysia.

**Effect of Soil Moisture Deficit Stress on Physiological and Biochemical Parameters of Banana Plants**

I. Ravi, M.M. Mustaffa and M. Mayilvaganan

*National Research Centre for Banana (NRCB-ICAR), Thogamalai Road, Thayanur (Post), Tiruchirapalli 620102, Tamil Nadu, India*

Water deficit is the major environmental factor limiting crop production throughout the world. Water deficit deviates plant metabolism from its normal pattern. The objective of the present investigation was to study the physiological and biochemical response of different banana genotypes to soil moisture deficit stress. The study was conducted with five genotypes, namely ‘Robusta’ (AAA), ‘Karpuravalli’ (ABB), ‘Saba’ (ABB), ‘Poovan’ (AAB) and ‘Nendran’ (AAB). Uniform planting materials were grown in 75 kg capacity concrete pots. Soil moisture deficit stress was imposed for 3 weeks by withholding irrigation, and control was maintained with regular irrigation to
field capacity. Sampling was done at weekly intervals and soil moisture status was monitored. Significant changes were observed in the tested genotypes after 3 weeks of stress in respect to Relative Water Content (RWC) and Membrane Stability Index (MSI), total sugars and free amino acids and other antioxidative enzymes. ‘Poovan’ recorded significantly higher RWC (55.79 %), MSI (46.83%) than the other four genotypes. The SOD and peroxidase activities were increased 2-3 fold in all the genotypes of unirrigated plants as compared with control plants. However, ‘Nendran’ and ‘Robusta’ recorded significantly higher activity of these enzymes compared to ‘Poovan’, ‘Karpuravalli’ and ‘Saba’. ‘Nendran’ and ‘Robusta’ plants recorded more senescent leaves (2.25) compared with ‘Poovan’ (1.05). There were no significant differences observed for leaf emergence rate and pseudostem girth between irrigated and unirrigated plants during the experimental period. Even if no new leaves are produced, maintenance of active leaves during soil moisture deficit stress period seems to be important. Among the tested genotypes, ‘Poovan’ appears to be more tolerant to soil moisture deficit stress, and RWC and MSI parameters may be used for screening soil moisture deficit stress.
Organising Committee

Convenors:
Dr. Jiang Zongyong
Dr. Yi Ganjun
Dr. Agustin Molina
Dr. Inge Van den Bergh

Committee:
Mr. Bai Xianjin
Mr. Lin Jianliang
Dr. Zhang Zongwen
Prof. Chen Weixin
Prof. Peng Hongxiang
Prof. Zhang Shaoxing
Mr. Liu Guanghua
Dr. Wei Yuerong
Prof. Huang Bingzhi

Secretariat:
Ms. Karen Lehrer
Mr. Chen Daoming
Ms. Lisbeth Barona

Treasurers:
Ms. Tina Aourai
Qin Suijia

Scientific Committee

Convenors:
Dr. Nicolas Roux
Dr. Yi Ganjun

Members:
Dr. Huang Bingzhi
Dr. Mike Smith
Dr. Rony Swennen
Dr. Wei Yuerong
Dr. Catur Hermanto
Dr. Li Chunyu
Dr. Agustin Molina
Dr. Alice Churchill
Dr. Zaag de Beer
Dr. Huang Xuelin
Dr. Inge Van den Bergh
Dr. Victor Galan-Sauco
Dr. H.P. Singh
Dr. Jin Zhiqiang