MEETING REPORT

Molecular tools for quality improvement in vegetatively propagated crops, including banana and cassava*

Banana and cassava play an important role as staple food in developing countries, contributing calories, nutrients, and to a limited extent, proteins to the diet. It is ironic therefore that relatively less attention has been given to genetic improvement of banana and cassava in comparison to other major food crops such as rice, wheat and maize. Most cassava and banana are grown by small holders for self-consumption and traded in the local market, providing income for the rural population in many low-income, food-deficient countries. In most of sub-Saharan Africa, where the green revolution had failed miserably, banana and cassava are providing food security for the people. Genetic improvement of these important crops will help directly to meet the UN Millennium Development Goals, particularly those related to health and nutrition, and the reduction of poverty and hunger. However, research in banana and cassava is largely under-funded.

The Food and Agriculture Organization (FAO) of the United Nations and the International Atomic Energy Agency (IAEA) have, in recognition of the paramount socio-economic importance for the tropics and for a number of their developing member States through their Joint FAO/IAEA Programme for Nuclear Techniques in Food and Agriculture, empanelled a Coordinated Research Project (CRP) to use innovative molecular approaches to address some critical production constraints to both crops. Both banana and cassava lack in the availability of genomics tools that could enhance crop improvement programmes. Also, in order to increase the yield that will be required to meet the expected population growth in Africa, Asia and Latin America, researchers must increase and stabilize yields by developing disease-resistant and stress-tolerant varieties. The FAO/IAEA Programme therefore addresses these issues by undertaking this CRP titled 'Molecular tools for quality improvement in vegetatively propagated crops, including banana and cassava'. The CRP emphasizes the application of induced mutations and new tools of functional genomics to solve the constraints of quality and related traits in banana and cassava. There are 12 research contract holders from the National Agricultural Research Systems (NARS) of Bangladesh, Brazil, China, Cuba, Ghana, India, Indonesia, Kenya, Mexico, Nigeria and the Philippines and five research agreement holders from the advanced laboratories in the Czech Republic and the United Kingdom. Two centres of the Consultative Group for International Agricultural Research (CGIAR), the International Centre for Tropical Agriculture (CIAT, its Spanish acronym), Cali, Colombia; and the International Network for the Improvement of Banana and Plantations (INIBAP) of Biodiversity International, Montpellier, France, are also participating as Research Agreement holders.

The first Research Coordination Meeting (RCM) was held at the headquarters of the IAEA in Vienna, Austria from 18 to 22 July 2005. At the end of this RCM India, also a centre of origin of banana, was selected as the venue for the next RCM. Thus the second RCM was hosted by the University of Kerala, Thiruvananthapuram.

The RCM was attended by 18 participants, including the Scientific Secretary and two observers: John Mba Chikelu Heslop-Harrison, the University of Leicester, UK and Uma Binita, National Research Centre for Banana, Tiruchirapalli, Tamil Nadu, India. The presentation and peer discussion of progress reports was followed by the presentation by participants, of the proposed work plan and discussions aimed at fine-tuning and/or streamlining proposed activities to tally with set objectives.

The RCM was inaugurated by K. Raghuraman (BARC, India). He noted that India has contributed greatly towards the genetic diversity of *Musa* species. In India, bananas are popularly known as 'kalpatharu' (meaning herb with all imaginable uses). Also cassava has become a staple food in South India. Hence the outcome of this CRP will have direct impact on the socio-economic development of the member nation.

The presentations of the research group working on banana started with Robert Miler (University of Catolica de Brasilia, Bazil). He presented the development of expressed sequence tags (ESTs) and resistance gene analogues (RGAs) related to resistance to *Musa fijiensis*. It is envisaged that the simple sequence repeat (SSR), EST and RGA-derived functional markers to be developed from these resources, in addition to the ESTs to be incorporated in a gene expression array, will lead to the identification of genes implicated in resistance to this fungal disease in *Musa*. Jorge Lopez Torres (Institute de Investigaicones en Viandas Tropicales, Cuba) presented activities relating to *in vitro*-induced mutagenesis, somatic embryogenesis from cell suspension cultures in *Musa*, and production of regenerants with minimal chimeras. The genetic stability of plants obtained via embryogenesis through embryogenic cell suspensions showed the possibility of using the new explants for developing somatic embryos. Jaroslav Dolezel (Laboratory of Molecular Cytogenetics and Cytometry, Czech Republic) have recorded significant progress towards the development of a cytogenetic *Musa* map using the BAC FISH strategy. Three repetitive units were mapped to a chromosome. Additionally, almost 600 repetitive DNA clones that represented various types of DNA repeats were selected for the assembly of a boutique DNA microarray which would lead to a better understanding of the genome organization in *Musa*. Ashalatha Nair (University of Kerala, Thiruvananthapuram) reported the progress made in Indian activities aimed at the development of di-haploid plants. Friable brown and white calluses and globular somatic embryos were obtained from anther cultures of different diploid genomes. The successful completion of this will arm *Musa* scientists with a tool that will facilitate the generation of homozygous starting materials for induced mutagenesis. Andrew James (Centro de Investigation Cientifica de Yucatan, Mexico) reported the development of conserved orthologous (COS) markers for drought stress tolerance-related and starch biosynthesis. Catabolism genes and their allelic diversity is at the core of the activities of the Mexican counterpart. Significant progress has been made in

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this through amplification of the desired genomic regions. The sequencing of these amplified regions is in progress.

The work by Golam Ahmed (Institute of Radiation Biology, Bangladesh) highlighted significant progress in the development of doubled haploid plants through banana anther culture. These plants have been potted and their ploidy levels will be determined using flow cytometry in due course. This development opens the way for exposing the anthers to mutagens and later generating di-haploid (equivalent to diploid) plants that would be homozygous for the different loci. The other option of generating di-haploid plants from the anthers of putative mutants would also enhance efficiency for induced mutagenesis in this crop.

Emma Sales (University of Southern Mindanao, the Philippines) presented her work on 'Molecular marker techniques for selection of induced mutation of banana with improved post harvest qualities'. Trude Schwarzacher (University of Leicester, UK) presented an in-depth over view on the 'Application of PCR and molecular cytogenetic tools to support breeding and exploitation of biodiversity in vegetatively propagated crops'. She has used IRAP as a marker for measuring genetic diversity in *Musa* species.

The section on the work on cassava started with Emmanual Okogbenin (National Root Crop Research Institute, Nigeria). He reported the preliminary evaluation of induced cassava mutants. The activities of the Nigerian counterpart indicated the development of variants with significantly elevated levels of dry matter and carotenoid contents.

John Beeching (University of Bath, UK) reported work on cDNA microarray and highlighted the central role of reactive oxygen species (ROS) together with enzymes and compounds that modulated them in the post harvest physiological deterioration (PPD) response in cassava. In addition, the significant changes in expression of pro- and anti-apoptotic genes during PPD suggested that PPD is also a senescence or apoptotic event. The tentative conclusion was that PPD is a ROS-mediated senescence phenomenon. The next stages will involve the test of this conclusion through the use of these genes in transforming cassava. Once established, molecular markers will be developed from these genes and used in routine assays of germplasm characterization or marker-aided selection. Zaochang Liu (Shanghai Academy of Agricultural Sciences, China) presented a protocol for the induction of somatic embryos from axiliary buds of a Chinese cassava genotype grown in Hainan Island, China. Somatic embryogenesis in cassava has proven to be recalcitrant, as there have been strong genotypic responses. It would be worthwhile therefore to establish these protocols for a wide spectrum of cassava genotypes spread over different agro-ecologies where the crop is cultivated. Luiz Carvalho (EMBRAPA, Brazil) presented the biochemical characterization of carotenoid and starch profiles of spontaneous mutant cassava landraces, and their inclusion in gene expression assays is contributing to the elucidation of the functions of genes. Additionally, the development of ESTs relating to these traits by this counterpart contributes to the identification of genes and eventual development of molecular genetic markers for assaying these traits. Elizabeth Okai Parkes (Crop Research Institute, Ghana) discussed her work on 'Adding value to cassava: using induced mutation breeding and marker assisted introgression of genes for delayed PPD and high quality starch into Ghanaian gene pools'. Two landraces were selected for *in vitro* culture and induction of mutation. John Ndung'u ng'ang'a (Kenyan Agricultural Research Institute, Kenya) was working to develop superior, well-adapted cassava varieties with high root quality traits through induced mutation and marker-aided breeding. Enny Sudramonowati (Research Center for Biotechnology, Indonesia) talked about improvement of Indonesian cassava through irradiation and molecular markers.

Two lectures relevant to the implementation of the CRP were delivered by John Heslop-Harrison on 'Association genetics' and by John Beeching on 'Good experimental methods'. This helps underscore the communal philosophy of the CRP mechanism and the implicit mentoring atmosphere it nurtures among member countries. The discussions were characterized by high level of synergies amongst the participants. This was evident in the probing to ensure not only that participants had not strayed from set goals, but also that the holistic goals would be achieved through identification and exploitation of the complementarities between the activities of different participants.

The proposed activities were treated in a similar manner, evaluated to ensure that the expected individual outputs would, as much as possible, dovetail with those of other participants and as a community lead to a successful CRP implementation. To achieve this part of the exercise, the meetings were frequently broken into working groups along crop lines, banana and cassava. Additionally, field trips to Indian R&D facilities of critical importance to the CRP were conducted. The choice of India as a venue for the meeting was predicated upon the country's possession of abundant genetic resources for one of the CRP crops, *Musa*. A field trip was therefore undertaken to the Banana Research Station, Kerala Agricultural University, Thrissur. This trip provided an opportunity to interact with the scientists at the station with the possible result of eventual exchange of germplasm and relevant information for genetic improvement of the crop and other research activities. Thiruvananthapuram is also home to the Central Tuber Crop Research Institute, with cassava as one of its mandate crops. Participants at this RCM also visited this institute, again providing an opportunity for forging collaborative initiatives. Participants also visited the Department of Botany, University of Kerala, and Agriculture University, Vellanikkara, providing an opportunity to interact with Indian researchers.

Genomic resources exist for banana and cassava, but they are yet to be widely deployed in breeding and integration with germplasm enhancement through induced mutagenesis. The goal of this CRP is the development of well-characterized mutants, advanced and pre-breeding lines, applicable data about genes, and genomic tools that can be combined with field-based breeding methods to increase the efficiency and reduce the time for improvement of multiple quality and related traits in banana and cassava. The RCM, as envisioned in the CRP mechanism, provided the participants and the sponsoring organizations with a veritable opportunity to review progress and plan for future activities for this project. Also, the mentoring aspect of a CRP, though subtle, was a major driving force for the successful implementation of collaborative activities and was obvious also in the interactions amongst the counterparts.

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