BANANA RESEARCH IN THE FAO/IAEA AGRICULTURE AND BIOTECHNOLOGY LABORATORY

R. MORPURGO, R. AFZA, H. BRUNNER, N. ROUX,
G. GRASSO, K.S LEE, M. VAN DUREN, F.J ZAPATA-ARIAS
Agriculture and Biotechnology Laboratory,
International Atomic Energy Agency,
Seibersdorf

Abstract

The primary activity of the Agriculture and Biotechnology Laboratory on banana has been to develop and transfer mutation techniques using nuclear and related biotechnology, provide training and mutagen treatment services and technical advice to the Member States. The complex genetic nature and lack of seed formation do not allow conventional breeding of *Musa* varieties. The FAO/IAEA laboratory has developed *in vitro* techniques to induce mutations, minimize chimerisms, and rapid propagation of banana. The most commonly used method of propagation is rapid proliferation of axillary and adventitious buds from meristem tip culture. Somatic embryogenesis has been induced in clones with different genomic constitution; however, the low germination rate of somatic embryos is still a major constraint. Investigations have been carried out on enzymes associated with resistance to *Fusarium oxisporum* f. sp. *cubense*. Molecular methods based on DNA oligonucleotide and DNA amplification fingerprinting are being developed for genomic characterisation of species, cultivars and mutant clones.

1. INTRODUCTION

The Plant Breeding Unit of the FAO/IAEA Agriculture and Biotechnology Laboratory, in co-operation with the Plant Breeding and Genetics Section, Joint FAO/IAEA Division, provides scientific and technical backstopping in several areas of plant breeding. The special emphasis is on the development and transfer of mutation breeding techniques to Member States. The work aims at technology development and transfer through the following activities: i) research and development of mutation techniques using nuclear and related biotechnological methods, ii) training of scientists, and iii) provision of mutagen treatment services and technical advice.

The difficulties encountered in the improvement of vegetatively propagated crops through traditional cross-breeding require the development of alternatives methods. In this context, mutation breeding in combination with related biotechnology can plays a major role. The complexity of *Musa* genetics illustrates the need for a more sophisticated system to support both conventional cross-breeding and mutation induction programmes. This report provides a general survey of the activities of the Plant Breeding Unit in the development of an integrated breeding approach for the improvement of bananas and plantains. Most of the work and achievement presented in here should be considered the result of co-operative efforts between a number of laboratories in the developing and developed countries. Therefore, a due acknowledgement and appreciation of the joint efforts toward a common goal is recognized.

2. RESEARCH ACTIVITY IN THE LABORATORY

Bananas and plantains (*Musa* spp.) are among the world's most important crops. They are staple food for millions of people throughout the developing world and an essential source of income in some of them. The complex genetic background, lack of seed formation and distortion of segregation in diploids and triploids are some of the factors that hamper the

development of new *Musa* varieties with the required resistance and quality characteristics. Studies on the development of banana and plantains were initiated at the FAO/IAEA Laboratory in 1985 as part of Joint FAO/IAEA Division programme.

2.1. Mutation Breeding and *In Vitro* Techniques

Any breeding programme relies on the availability of genetic variation, well characterised parental material, and the possibility to rapidly screen traits among recombinants or in large mutagenised populations. The mutation breeding system developed at the FAO/IAEA laboratory is based on *in vitro* techniques for inducing mutations and avoiding genetic chimerisms, and propagation of the desired variants. The most commonly used method of propagation is rapid proliferation of axillary and adventitious buds from meristem-tip culture. This also allows the checking of viral contamination. The laboratory has now capability to check for the presence of the common viral diseases by using ELISA techniques. The material received from counterparts are tested for the presence of pathogens.

The development of somatic embryogenesis, using somatic tissue explants was a breakthrough in the development of a novel method. It opened the possibility to induce somatic embryogenesis in clones with different genomic constitution. However, a major constraint is the low germination rate of somatic embryos. Generally, somatic embryos have a strong tendency to form roots. Thus, there is a need to fine tune the germination medium and to identify the possible blockage in the somatic embryogenesis pathway. Nevertheless, this method offers new opportunities of breeding new cultivars. The system allows production of embryogenic suspension cultures for mutation induction. The unicellular system enables selection for various stresses both abiotic and biotic, and could be used in breeding procedures for *in vitro* selection and polyploidy induction.

2.2. Screening for resistance to *Fusarium* wilt

Breeding for disease resistance is a major goal in *Musa* improvement. In spite of the recent success in the development of resistant clones through cross-breeding in Honduras and somaclonal variation in Taiwan, the development of methods based on easily detectable markers should be considered a priority to shorten the lengthy field screening procedures. During the past five years, a major effort in the Plant Breeding Unit has been to work out a system for early selection of disease resistance in banana, and to identify biochemical markers linked to this trait.

Shoot tips cultures from banana clones susceptible or resistant to *Fusarium oxisporum* f. sp. *cubense* (FOC) Race 1 and 4 were grown *in vitro* in the presence of different concentration of fusaric acid and fungal crude filtrates or inoculated with conidial suspension of FOC to assess correlation between *in vivo* or *in vitro* behaviour. Explants were susceptible to both the filtrate and fusaric acid irrespective to their known field resistance/susceptibility response. No clear linkage between *in vivo* and *in vitro* behaviour was observed. The results suggested that the use of crude filtrate or non-host specific toxin to screen for resistance to FOC in *Musa* was not feasible. When peroxidase activity was used as a parameter to discriminate between susceptibility and tolerance, results were in good agreement with the field response of host plants to pathogens. Early enzymatic activity increased in the incompatible host-pathogen interaction but not in the compatible interaction. Studies were carried on other enzymatic systems involved in plant resistance, especially on chitinase. Forty-two clones were screened for chitinase activity. Further studies were conducted on the

behaviour of this enzyme; the laboratory is now trying to clarify the role of chitinase in banana resistance.

2.3. Genetic markers and molecular biology

The characterisation of breeding material and its stability represents a major concern in any improvement programme. DNA oligonucleotide and DNA amplification fingerprinting (DAF) were used for genomic characterisation of different species, cultivars and a mutant clone. Both the oligonucleotide and DAF were somatically stable, i.e., did not exhibit any difference between tissues of the same plant and between individuals of the same clone. A unique fingerprinting was found for each clone, and therefore could be used to discriminate between accessions or to characterise the parental material and mutants. The use of Random Amplified DNA (RAPDs) is being investigated for the analysis of homogeneity among near isogenic lines to identify genes for resistance. For this, PCR cloning of specific genes is being pursued to clarify their specific function in the host-parasite interactions. Genetic transformation of *Musa* represent yet another opportunity to develop new genotypes. Transgenic 'Grand Naine' were obtained by the *Agrobacterium*-mediated and high-velocity microparticle bombardment transformation. Breeding of banana and plantains is thus being complemented through the development of techniques that would enhance breeding efficiency. The Plant Breeding Unit in collaboration with several institutes is refining the technology and its transfer through training.