Plant breeding: Induced mutation technology for crop improvement

Scientists at the IAEA's Seibersdorf Laboratories are helping breeders to develop crops having more desirable traits

by F.J. Novak and H. Brunner

All present forms of life are the product of three factors:

- mutation, the fundamental source of heritable variation,
- environmental factors, which influence the selection of those mutations that survive and reproduce, and
- time, during which the genotype and environment constantly interact and evolutionary change is realized.

Genetic variation found in nature does not represent the original spectrum of spontaneous mutations. Rather, this is the result of genotypes recombining in populations and continuously interacting with environmental forces.

Green plants are the ultimate source of resources required for human life, food, clothing, and energy requirements. Prehistoric people, who depended on their skills as hunters, drew upon abundant natural vegetation to collect nutritious and nonpoisonous fruits, seeds, tubers, and other foods. As human populations increased, greater and safer supplies of food had to be found, and gradually production systems based on plant domestication were developed.

The domestication of crops historically has been influenced by ecological and agricultural conditions, as well as by food gathering preferences. Genotypes that have adapted to a wide range of climatic and edaphic conditions typically have been selected for cultivation. The achievement of higher yielding crops facilitated population growth, sedentary settlements, and further development. Which crops were domesticated depended not only on the number of seeds or the size of fruits, but also on taste, palatability, and other factors.

Only a small fraction of the world's approximately 200 000 plant species have been found suitable for domestication; humans have used about 3000 of these for food, fibre, spices, etc., with 200 ultimately domesticated as crops. Today, only 15-20 of these are food crops of major importance.

The means of developing new plant varieties for cultivation and use by humans has come to be called plant breeding. Early on, it primarily involved selection, the choice between good and bad plants. People learned not to eat all the "best fruit" but to plant the seed from some of them.

Genetics became a fundamental science of plant breeding after the Moravian monk J.G. Mendel discovered the laws of heredity in the mid-19th century. Plant breeding further advanced when the methodology of hybridization was developed. Its aim was to combine various desirable properties of many plants in one plant, instead of just choosing between good and bad plants. This method, often supplemented by germplasm derived from induced mutation, has become the most common one for breeding plants through sexual reproduction.

However, some crops—including bananas, apples, cassava, and sugar cane—reproduce vegetatively, especially those that are fully sterile without seeds. For this important group, alternative approaches had to be developed, namely techniques of manipulation with somatic tissue: mutation breeding and biotechnology.

Mutation breeding

Plant breeding requires genetic variation of useful traits for crop improvement. Often, however, desired variation is lacking. Mutagenic agents, such as radiation and certain chemicals, then can be used to induce mutations and generate genetic variations from which desired mutants may be selected.

Mutation induction has become a proven way of creating variation within a crop variety.

Dr Novak is Head of the Plant Breeding Unit at the IAEA's Seibersdorf Laboratories, and Dr Brunner is a senior scientist in the Unit.

One natural evolutionary product of genetic variation: a mutant of dwarf coconut palm.

It offers the possibility of inducing desired attributes that either cannot be found in nature or have been lost during evolution. When no gene, or genes, for resistance to a particular disease, or for tolerance to stress, can be found in the available gene pool, plant breeders have no obvious alternative but to attempt mutation induction.

Treatment with mutagens alters genes or breaks chromosomes. Gene mutations occur naturally as errors in deoxyribonucleic acid (DNA) replication. Most of these errors are repaired, but some may pass the next cell division to become established in the plant offspring as spontaneous mutations.

Although mutations observed in a particular gene are rare, there are probably 100 000 genes in a cell of a higher plant. This means that every plant may carry one or more spontaneous mutations into the next generation. Gene mutations without phenotypic (visible) expressions are usually not recognized. Consequently, genetic variation appears rather limited, and scientists have to resort to mutation induction. There are no other economic ways of altering genes, except to wait a long time for spontaneous mutations to occur.

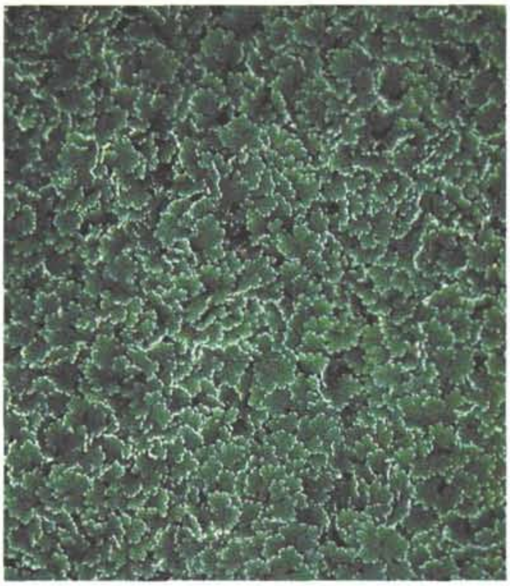
Artificial induction of mutations by ionizing radiation dates back to the beginning of the 20th century. But it took about 30 years to prove that such changes could be used in plant breeding. Initial attempts to induce mutations in plants mostly used X-rays; later, at the dawn of the "Atomic Age", gamma and neutron radiation were employed as these types of ionizing radiations became readily available from newly established nuclear research centres.

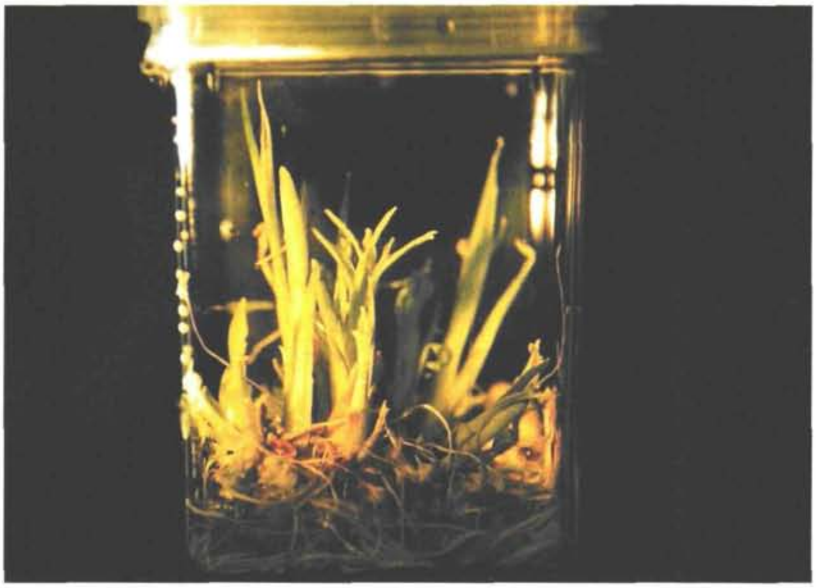
Major efforts were devoted during this initial phase of mutation induction to define optimal treatment conditions for reproducibility. Research focused on changing "random" mutation induction into a more directed mutagenesis to obtain more desirable and economically useful mutations. However, it did not lead to the desired alterations in the mutant spectrum. Limitations were the concomitant increase of plant injury with increasing radiation dose and the low frequency of economically useful mutations. This led scientists to search for potentially better mutagens. As a result, new methods of radiation treatment, as well as chemical agents with mutagenic properties, were found.

Plant biotechnology

Breeding for improved plant cultivars is based on two principles: genetic variation and selection. The process is extremely labourious and time consuming with high inputs of intellectual and manual work. *(See box.)* However, the development of plant cell and tissue culture over the last 20 years has made it possible to transfer part of the breeding work from field to laboratory conditions.

Extensive research has resulted in new areas of plant breeding, namely "plant biotechnology" and "genetic engineering". They are based on cellular totipotency, or the ability to regenerate whole, flowering plants from isolated organs (meristems), pieces of tissue, individual cells, and protoplasts. The isolated plant parts are aseptically grown in test tubes on artificial media





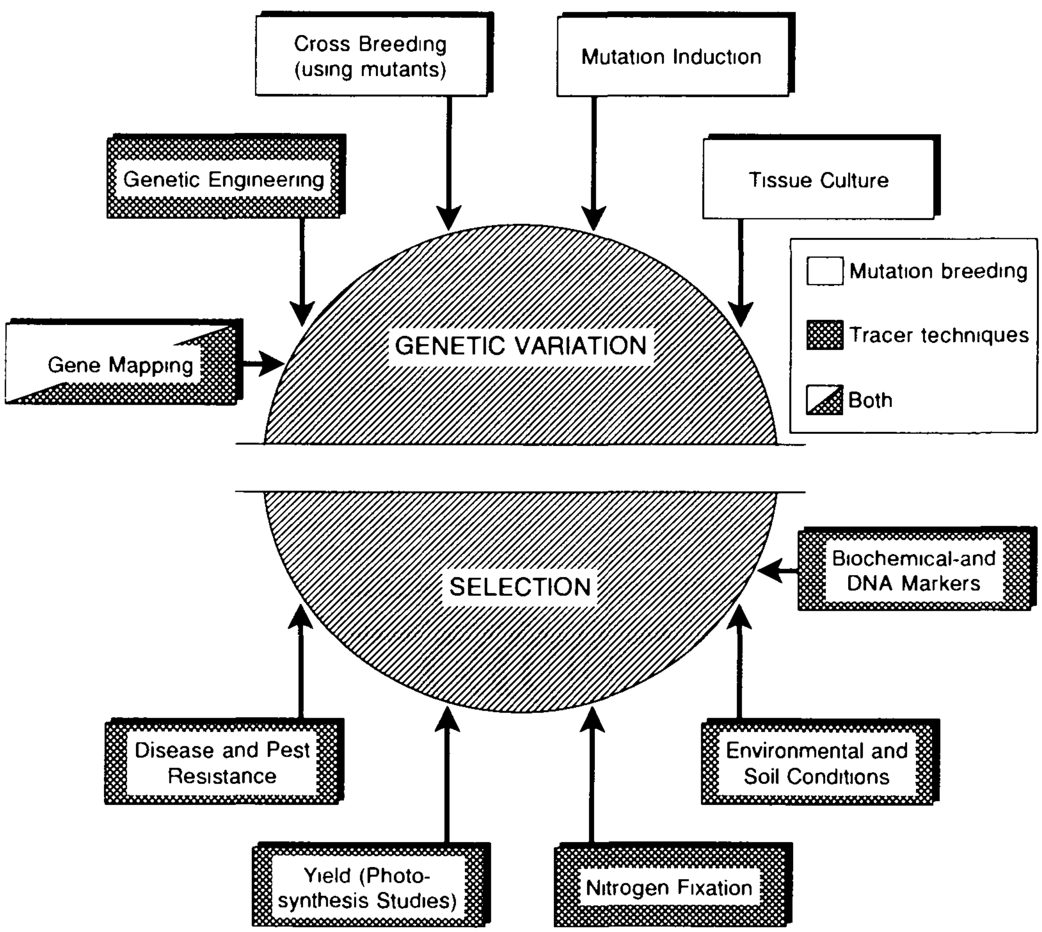
Some tools and products of plant breeding *(clockwise from top left)*: **a mutant of paddy rice induced by ionizing radiation; yams and other root and tuber crops can be genetically improved by mutation breeding; tissue culture and in vitro mutagenesis are basic methods of biotechnology for improving crops; "Golden Haidegg", an apple mutant with improved market value, was induced at the Seibersdorf Laboratories by irradiation of cuttings from "Golden Delicious" apples; mutation breeding has improved the tolerance to environmental stress of Azolla, a water fern used as biofertilizer in rice paddies.**

General scheme of mutation breeding

Breeding a new variety of crop takes anywhere from 12 to 15 years of intensive effort The steps include:

Generation	Characterization
	Seeds, pollen, vegetative parts, or tissue cultures treated by physical (radiation) or chemical mutagens.
$M_1(M_1V_1)$	Plants grown from treated seeds (M_1) or vegetative propagula (M_1V_1).
$M_2(M_1V_2)$	Population of plants grown from seeds (M_2) or vegetative parts (M_1V_2) harvested from M_1 or M_1V_1 respectively. Selection of desired mutants may start in this generation or later.
M_3 - M_8 (M_1V_3 - M_1V_8)	Continuing selection, genetic confirmation, mulitplication and stabilization of field performance of mutant lines.
Next 2 - 3 generations	Comparative analyses of mutant lines during different years and in different locations.
Next 2 - 3 generations	Official testing before release as a new variety.

Applications of nuclear techniques in plant breeding



Crop improvement is based on two basic principles: genetic variation and selection. Serving as invaluable tools are mutagenic irradiation and isotope tracer techniques, which are incorporated into the various breeding methods.

of known chemical composition (*in vitro* culture). Under strictly controlled conditions, they form plantlets that subsequently can be transferred to soil where they grow to maturity.

Tissue culture has been exploited commercially for micropropagation of disease-free stocks of horticultural plants (strawberry, potato, and ornamentals, for example). *In vitro* techniques also are useful in various steps of the breeding process, such as germplasm preservation, clonal propagation, and distant hybridization.

Radiation mutation breeding and isotope techniques, combined with tissue culture, have made a significant contribution to plant breeding. They have introduced new techniques for inducing genetic variation, by improving selection technology, and by accelerating breeding time. *(See box.)*

Other methods, known as anther, or pollen culture, make it possible to regenerate plants from male gametes with half the number of chromosomes—haploids. Compared to plants with full chromosomal content (diploids), the use of haploids in mutation breeding is advantageous since it allows detection of mutations immediately after their induction. Haploid methods have proven to significantly speed up the breeding of new varieties of rice, barley, and vegetables, for instance.

Genetic engineering procedures allow the transfer of genetic material (DNA) from the cell of one species to that of another genetically unrelated organism. For example, a piece of DNA from a bacterial cell may be integrated into the genome of a plant cell to form a transgenic plant. The new DNA (gene) expresses itself in the plant phenotype regenerated from the transgenic cell. Nuclear techniques, based on nucleic acid bases labelled by isotopes, are employed in genetic engineering, to identify and isolate suitable genes for transfer; as delivery systems to introduce genes into recipient cells; and to detect new genetic material in recipient organisms.

Genetic engineering already has resulted in the production of plants with new desirable traits, such as insect resistance, virus disease resistance, and better ripening properties. However, early enthusiasm is being tempered by the growing discussion of the potential hazards of releasing transgenic plants to the environment.

Another issue that has emerged concerns the commercialization of this technology, and the access of developing countries to it. Recent developments in plant biotechnology have resulted in enormous capital investments and in a concentration of highly qualified human resources into the commercial sector of many industrial countries. In the process, scientific knowledge and its technological applications increasingly are becoming the subject of commercial legislation, involving patents, industrial secrecy, and licensing policy. As a result, developing countries have experienced difficulties in gaining access to biotechnological results and their applications in national programmes.

In this context, specialized agencies of the United Nations—including the Food and Agriculture Organization (FAO), United Nations Educational, Scientific and Cultural Organization (UNESCO), United Nations Industrial Development Organization (UNIDO), and IAEA—are playing an important role. Programmes have been set in place whose main concerns are to identify and transfer appropriate biotechnologies, and to train personnel, in developing countries. In this way, national capabilities in research and development are being strengthened in this field.

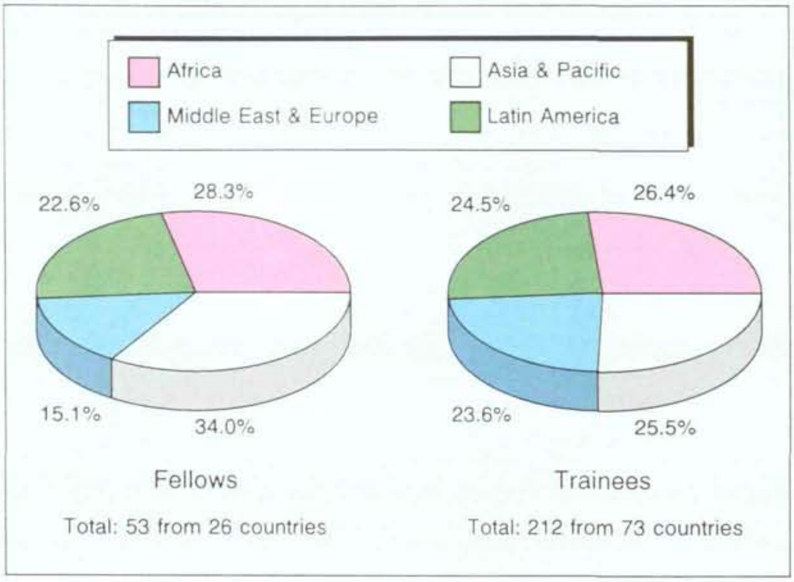
Plant breeding for sustainable agriculture

Plant breeding and biotechnology contribute greatly to environmentally friendly agriculture. The focal point is the establishment of a comprehensive gene pool for crop improvement.

New technologies have been developed to stop the loss of biodiversity of plant species. Tissue culture technology in cassava, banana and plantain, potato, yam, sweet potato, and coconut, for example, is becoming the preferred method for germplasm preservation and for the international exchange of clonal material. Molecular marker techniques are applied for the classification and genetic analysis of cultivars and related wild species. Plant breeding methods such as cross breeding, *in vivo*, and *in vitro* mutation breeding very often include nuclear components. They are employed for the enhancement of genetic resources by mutation induction, recombination, and selection.

Most breeding projects are directed towards developing new plant cultivars that exhibit greater disease resistance and tolerance to pests than the original plant. Such varieties decrease dependence on agro-chemicals, which is a basic feature affecting sustainable agricultural development in industrialized and developing countries alike. Resistance breeding may help to avoid the epidemic situation in plantation crops. These include the swollen-shoot virus disease epidemic on cocoa in Ghana, and Panama disease on banana in several tropical and subtropical areas.

Sustainable plant breeding today faces some major challenges. They specifically relate to breeding plants for improved nitrogen fixation and plants with better capacities to utilize nutrients. Integrated research involving both soil



Training activities in the Plant Breeding Unit of the Seibersdorf Laboratories, 1982-92

scientists and plant breeders already has identified desirable genotypes in grain legumes (soybean, garden bean) and other plant species, including trees.

For many developing countries, breeding crops for tolerance to salinity and acidity in soils is of high priority. Current breeding strategies (including mutation induction and *in vitro* selection) have clearly been successful in incorporating degrees of tolerance in different species. The use of genetic engineering for creating environmental stress-resistant plants will depend on the identification of specific genes which contribute to the adaptation to specific stress environments.

In tropical countries, agriculture practices have maintained the yield level of different crops through "intercropping" instead of by increased monocrop cultivation. Breeding crops for multiple functions—such as biomass production, improved soil and water practice, and composting—is a desirable support of sustainable agriculture in developing countries. The mixed planting of a main crop with specific cover crops (e.g. forage legumes or grasses) minimizes the use of herbicides.

Radiation service statistics, 1967-92

Treated samples	20 329
Treated species	217
Treated cultivars	1 134
Recipient Member States	108
Seed samples	17 872
Vegetatively propagated plants	1 046
Cobalt-60 gamma treatments	14 382
Fast neutron treatments	5 416
Other mutagen treatments	531

Note: Examples of major plant species treated include: cereals (rice, wheat, barley, tricilale, millet, tef); legumes (soybean, peanut, common bean, cowpea, mungbean); root and tuber crops (cassava, yam, cocoyam, potato); fruits (citrus, apple, apricot, peach, grape vine); ornamentals (chrysanthemum, antirrhinum, achimenes, tulip); and others (rape, sesame, amaranth, quinoa, niger).

Role of the Seibersorf Laboratories

The Plant Breeding Unit of the IAEA Laboratories at Seibersdorf was set up in the mid-1960s to support the Joint FAO/IAEA Division's programme of genetic crop improvement. Nuclear techniques in plant breeding are developed and transferred to countries by research and development in mutation breeding and related biotechnological techniques, training scientists from developing countries, and providing irradiation services and technical advice.

Initial research in the Plant Breeding Unit focused on the development of mutation induction methods with ionizing radiation and chemical mutagens. The aim was to achieve high mutagenic efficiency, i.e., a high frequency of desirable mutations at minimal plant injury and the highest possible reproducibility. This required a definition of radiation source characteristics in terms of dose homogeneity and precise assessment of absorbed dose in biological targets by appropriate dosimetry. Irradiation of seeds with gamma rays and neutrons was commonly done, given the ease of handling, the simple standardization of factors which modify radiation sensitivity, and good reproducibility. The establishment of methods for controlling oxygen-dependent effects in the radiobiological response to electromagnetic radiation was a major achievement. The Laboratory actively contributed to standardizing neutron irradiation of seeds in nuclear reactors by developing special facilities for this purpose. These were known as SNIF, for Standard Neutron Irradiation Facility for swimming-pool-type reactors; and as USIF, for Uranium Shielded Irradiation Facility for Triga-type reactors.

This research was the basis for the IAEA Laboratories' worldwide seed irradiation service using fast and thermal neutrons at a high-dose precision and reproducibility of induced effects. Moreover, efficient and accurate treatments of seeds with chemical mutagens, mostly alkylating agents and azides, were developed with the aid of isotope-labelled compounds and compared with mutation induction by ionizing radiation. The Unit has undertaken supportive research on mutation breeding in cereals, pulse crops, industrial crops, and vegetatively propagated crops.

As each crop species has a variable reproductive capacity (number of progenies per plant) and a specific system of reproduction (self- or cross-pollinated sexual reproduction or asexual propagation), a universal breeding approach cannot be developed and species-specific procedures have to be applied. Most vegetatively or asexually propagated species are difficult to improve genetically by conventional cross- and mutation breeding methods. These breeding problems can be more easily resolved by using biotechnology in combination with mutation induction, and the Unit initiated *in vitro* mutation breeding activities during the mid-1980s. Several tropical food crops of great importance to the food security of developing countries were chosen as the main focus of R&D and training activities in biotechnological plant breeding at the IAEA Laboratories.

Research and development activities

The Unit provides focused support to the FAO/IAEA's co-ordinated research and technical co-operation programmes. Assistance is provided to numerous projects in terms of expertise for building facilities for plant tissue culture and mutagenic treatment, for quality control of dosimetry of mutagenic irradiation, and for the development and transfer of nuclear technologies for plant improvement.

Ongoing R&D includes the application of nuclear methods and associated advanced techniques, such as *in vitro* culture and molecular genetics, to improve the production of a wide range of crops through mutation breeding. The development of biotechnological methods for breeding vegetatively propagated crop plants of major importance in developing countries has a high priority.

Currently, the following R&D areas are being pursued:

- ***Somaclonal and mutagen induced variation.*** Systematic studies are being conducted to compare the genetic variation caused by tissue culture (somaclonal) variation with that induced by irradiation and chemical agents. Genetic variation is being studied among maize plants derived from *in vitro* cultured material via somatic embryogenesis. This is being done to assess the nature of somaclonal and induced variation and its potential for use in practical breeding.

- ***Mutation induction and breeding technology for banana and plantain.*** Low genetic variation and sterility handicap genetic improvement of banana and plantains *(Musa spp.)* by conventional breeding techniques. Shoot-tip culture and *in vitro* plant regeneration are being investigated for use in mutation induction and mutant selection. Somatic embryogenesis and plant regeneration from cell suspensions of *Musa* are used to develop somatic cell manipulation procedures for banana and plantain breeding. Methods of screening such plants for resistance to Panama disease are studied in tissue culture, and biochemical markers (peroxidase) are applied for the identification of tolerant genotypes. DNA markers are used for identifying mutants and characterizing cultivars and species of *Musa*. Mutant clones identified at the Seibersdorf Laboratories are tested in the field in tropical countries.

- ***Mutation breeding to improve the tolerance to environmental stress of Azolla.*** *Azolla* is a small aquatic fern that lives in symbiotic relationship with the nitrogen-fixing cyanobacterium *Anabaena*. Under suitable field conditions *Azolla* can double its weight every 3-5 days. The *Azolla-Anabaena* symbiotic system provides green manure for flooded crops, particularly rice. Induced mutagenesis has produced *Azolla* variants tolerant to high salinity, toxic aluminium levels, and/or to herbicides. Tolerant plants are being investigated under field conditions to confirm that heritable changes cause the increased tolerance to environmental stress.

- ***Methods of mutation induction and breeding of tropical root and tuber crops (cassava and yam).*** Cassava and yam are among the most important staple food crops of the lowland tropics. Mutation breeding technology is being developed to increase variation in plant stature, cyanide content, disease, and pest resistance. *In vitro* techniques are used for the propagation of healthy plants and improved clones. Somatic embryogenesis is being developed for cassava and yam improvement through *in vitro* mutagenesis and later on by somatic cell manipulation. Mutant and polyploid clones are prepared for field testing in Member States.

- ***Tissue culture in cocoa as a system for more efficient mutation breeding.*** Attempts to breed cocoa for disease resistance have yielded very limited success. A major constraint is that little variation exists in currently available cultivars. Somatic embryogenesis is being developed for propagation of desirable genotypes and, through *in vitro* mutagenesis and pollen mutagenesis, is being applied for induction of virus-resistant cocoa trees in Ghana.

Plant breeding research at the Seibersdorf Laboratories is directly problem- and client-oriented. Many positive results of scientific work have been achieved by junior scientists from developing countries during their assignments under the IAEA's fellowship training programme. Local cultivars and genetic material from tropical countries are brought to the Seibersdorf Laboratories, transferred to tissue culture conditions and used for experimental work. Protocols and techniques that are specifically developed for a crop and a particular genotype are then directly used in national programmes. Additionally, breeding material originating from mutant lines and clones which are ready for field testing are dispatched from Seibersdorf to developing Member States in support of their breeding programmes.

Training of plant breeders

Training in plant breeding represents the most active component of technology transfer at the Seibersdorf Laboratories. For 20 years the Plant Breeding Unit has supported the Agency's fellowship programme and organized interregional training courses. Training activities are closely connected with R&D efforts on crop plant improvement and the application of nuclear techniques in breeding. *(See graphs.)* During a period of three to twelve months, fellows usually work with radiation or chemical induced mutagenesis in plant species cultivated in their home countries. Whenever possible, training of small groups of two-to-five fellows is organized for solving common problems. The experiments are individually designed to assure that laboratory techniques and results will be directly applicable upon return to the home institute.

As a result of their work, fellows have produced numerous scientific publications in internationally recognized journals and symposia proceedings. Very often, as continuation of a fellowship in Seibersdorf, fellows participate in co-ordinated research and technical co-operation projects of the IAEA.

The FAO/IAEA Interregional Training Course on "Induction and Use of Mutations in Plant Breeding" has been held at the Seibersdorf Laboratories since 1982. Twenty participants from different Member States of FAO and IAEA are admitted annually to this intensive training course that usually lasts 6 to 8 weeks. Through lectures, laboratory exercises, field experiment evaluations, seminars, and excursions, participants are made aware of the latest advanced mutation techniques and biotechnological and molecular biology methods for crop improvement. Special training is given in the safe handling of radiation sources, radioisotopes, and particularly hazardous mutagenic chemicals. At the end of each course, participants are able to discuss and evaluate the potential role of induced mutations and advanced biotechnologies in their national breeding programmes for specific crop improvement of cereals, legumes, oil crops, forages, vegetables, fruits, root and tuber crops, palms, rubber, and other plants.

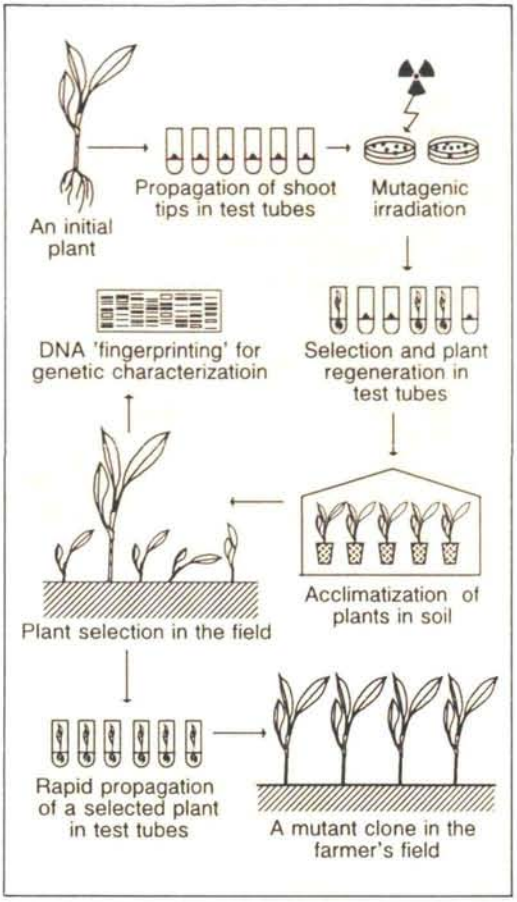
Support for national programmes

A radiation treatment service is provided at no cost to FAO and IAEA Member States to foster the application of nuclear techniques in crop improvement programmes and to render direct support to plant breeders in developing countries. Mutagenic treatment is applied to seeds, corms, tubers, scions, cuttings, and tissue cultures ("*in vitro* materials") with precise doses of gamma and fast neutron radiation. The doses are carefully calibrated to assure reproducible effects. Users of the service are requested to report on the objectives of the applied mutation breeding project and to provide an adequate material (population size) to ensure a high probability for mutation induction of desired characters. Moreover, a prior radiosensitivity test in a greenhouse is frequently performed to assess useful radiation doses for the great variety of biological samples in mutation breeding. The treated materials are dispatched with a detailed irradiation protocol and with the request to report on the induced radiation effects in the first and second mutation generation. This feedback is required to improve radiosensitivity estimates of species and cultivars from different environments.

Over the last 25 years, the Unit has provided radiation services on more than 20 000 samples from the majority of Member States from the FAO and IAEA. *(See table.)* Most of these were seed samples which were irradiated with cobalt-60 gamma rays.

Recently, however, requests for mutagen treatment of *in vitro* materials and for fast neutrons have become more frequent. This reflects the increasing importance of biotechnology and molecular genetics in plant improvement programmes.

Less than 80 mutant varieties were officially released before the start of irradiation services. Over the past quarter century, more than 1500 cultivars of crop plants and ornamentals with significantly improved attributes — increased yield, improved quality, higher market value, disease resistance, and/or stress tolerance — have been released. Some of these mutant varieties were derived from radiation services provided by the Seibersdorf Laboratory. ❐



A banana plant developed by mutation breeding using ionizing radiation. *At left*: The schematic represents a banana mutation breeding system.

Breeding hardier bananas

Bananas, plaintains, and cooking bananas are different cultivars and species belonging to the botanical genus *Musa*. Banana "trees" are actually big herbs which produce fruit that are one of the most important foods for hundreds of millions of people in developing countries. The world's production is more than 70 million tonnes per year and about 90% of the total harvest is used as food for domestic consumption. The banana industry generates an income of about US $1.7 billion annually for exporting developing countries.

The cultivation of bananas and plantains is seriously threatened by several diseases caused by pathogenic fungi, bacteria, viruses, and nematodes. Some of them may be controlled by pesticides; however, the most epidemic pathogen, Fusarium, is a soil borne fungus which causes Panama disease. There is no effective chemical control against the spread of this fungus in infested soil. Panama disease has devastated several hundred thousand hectares of banana plantations in Central America and created serious problems in Africa where many people are dependent on plantains and cooking bananas as part of their staple diet. The only way to resolve this problem is to breed varieties having disease resistance.

The world's production of bananas is based on a very limited number of genetically unimproved clones that were selected and domesticated from nature. Although cross breeding has contributed a little to banana breeding, the most important varieties are entirely sterile and therefore impossible to improve by conventional breeding techniques.

Research on the induction of mutations in bananas by exposing them to radiation and supporting tissue culture techniques was initiated at the Seibersdorf Laboratories in 1985. Shoot tips were isolated from several economically important banana and plantain cultivars and micropropagated on artificial media in test tubes. Several types of mutagenic irradiation (gamma rays and fast neutrons) were applied on actively growing cells of apical shoot tips which were regenerated into plants. This research resulted in the development of mutant clones of the most important cultivar of the dessert banana, "Grand Nain". These varieties are now being tested in several countries for agronomic performance such as yield, quality of fruit, and earlier harvest.

The Seibersdorf Laboratory supports a co-ordinated research programme on breeding for improvement of *Musa* crops and assists several technical co-operation projects in establishing national breeding programmes in Colombia, Panama, Costa Rica, Cuba, Ghana, Malaysia, and Thailand.

Recent developments in molecular biology have made it possible to characterize plant genomes and to identify markers for practical use in plant breeding. Genetic "fingerprinting" of banana cultivars and mutants opens new perspectives for breeding these genetically "recalcitrant" crops which are of such vital importance to people in developing countries.