

RADIATION TECHNIQUES IN CROP AND PLANT BREEDING MULTIPLYING THE BENEFITS

BY BEANT S. AHLOOWALIA

World food production is based on growing a wide variety of fruits, vegetables, and crops developed through advances in science. Plant breeders have produced multiple varieties that grow well in various types of soils and under diverse climates in different regions of the world.

Conventionally, this is done by sexual hybridization. This involves transferring pollen from one parent plant to another to obtain hybrids. The subsequent generations of these hybrids are grown to select plants which combine the desired characters of the parents.

However, another method exists by which the genetic make-up of a given plant variety can be changed without crossing with another variety. With this method, a variety retains all its original attributes but is upgraded in one or two changed characteristics. This method is based on radiation-induced genetic changes, and is referred to as “induced mutations”.

During the past thirty years, more than 1800 mutant varieties of plants have been released, many of which were induced with radiation. Plant tissue and cell culture (also called *in vitro* culture) in combination with radiation is a powerful technique to induce mutations, particularly for the improvement of vegetatively propagated crops. These crops

include cassava, garlic, potato, sweet potato, yams, sugarcane, ornamentals such as chrysanthemum, carnation, roses, tulips, daffodil, and many fruits (e.g. apple, banana, plantain, citrus, date palm, grape, papaya, passion fruit, and kiwi fruit). In some of these plants, either there is no seed set (e.g. banana) or the seed progeny produces plants which do not have the right combination of the desired characteristics. These techniques are also useful in the improvement of forest trees having a long lifespan before they produce fruit and seed.

This article briefly reviews advances in plant breeding techniques, with a view towards improving the transfer of technologies to more countries.

ACCELERATING THE PROCESS

In tissue and cell culture techniques, instead of propagating a plant from a seed or cutting, small pieces or parts of the plant are cultured under controlled environment and aseptic conditions on defined nutrient media, from which many miniature-sized plants can be obtained. *In vitro* culture of tissue has been often called “test-tube” culture of plants; however, the culture containers are not always glass test tubes. They can be much cheaper and versatile vessels, such as baby

food jars, jam jars, and disposable plastic cups used for coffee or yogurt.

Plant tissue culture — techniques either on their own or in combination with induced mutations and molecular techniques — are used to speed-up conventional plant breeding, and to rapidly multiply plants in a disease-free condition.

Plant cell and tissue culture methods are useful for mutation induction with radiation as well as chemicals. However, radiation is the preferred method to make genetic changes because of the ease in treating large populations, and the problems of handling and disposing of chemicals. Usually, mutations are induced in well-known and well-adapted plant varieties grown in a particular region. Selected and disease-free plants of these varieties are cultured *in vitro*, and irradiated with gamma or X-rays. The irradiated plants, tissues, or cells are then multiplied many times, also *in vitro*, from which full grown plants are produced. They are then grown in soil for selection of the desired types.

During the past two decades, several *in vitro* culture

Mr. Ahloowalia is a staff member of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

techniques have been developed. Among these are:

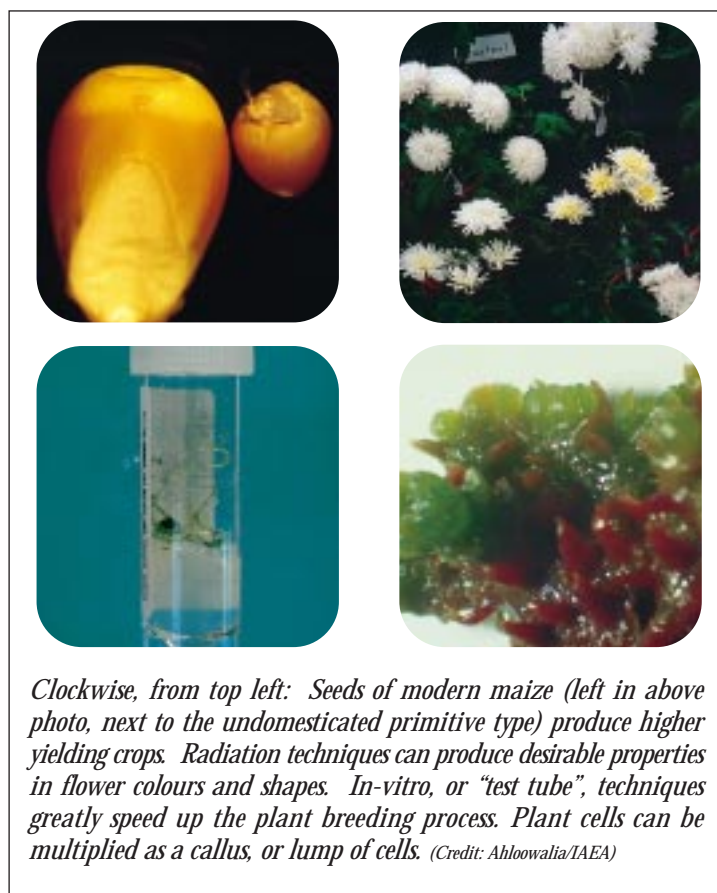
■ **Micropropagation.** Small pieces, three to five millimeters in size, are cultured from stems with terminal buds or from leaves. These proliferate into many buds and shoots which on transfer to another nutrient medium produce complete plants. This procedure is the most widely used method to produce millions of clones (exact copies) of plants such as banana, strawberry, potatoes and many ornamentals.

■ **Embryo rescue.** A tiny plant embryo, which normally would not survive during the normal course of development in the seed, is removed and cultured to give a full plant. This is widely used in the propagation of many orchids in which the seeds do not have sufficient food to support the growth of embryo to a full plant.

■ **Plant regeneration.** Many plants also can be produced by culturing cells (the building blocks of tissues). These are first made to grow into a lump (callus) or are grown as cell suspension cultures. Plants can be produced from these cells by changing the nutrient medium.

In some cases, it also possible to regenerate plants from protoplasts (naked plant cells from which cell walls have been removed). Protoplasts from different plant species (e.g. potato and tomato) can be fused to form somatic hybrids which would not be possible through sexual hybridization. Such hybrids are then irradiated to remove the unwanted genetic information of the parents.

The inactivation of cell nuclei or protoplasts with high doses



Clockwise, from top left: Seeds of modern maize (left in above photo, next to the undomesticated primitive type) produce higher yielding crops. Radiation techniques can produce desirable properties in flower colours and shapes. In-vitro, or "test tube", techniques greatly speed up the plant breeding process. Plant cells can be multiplied as a callus, or lump of cells. (Credit: Ahloowalia/IAEA)

of radiation for producing cybrids (somatic hybrids in which cytoplasm of one cell is fused with the nucleus of another cell) has been also used to produce new and novel gene combinations; for example to transfer cold tolerance of radish to rape seed.

■ **Anther and microspore culture.** By far, this has become the most important *in vitro* culture method used in conjunction with radiation-induced mutations to improve seed propagated crops. Unlike the body (somatic) cells which have two sets of genetic information, the pollen grains which are formed in anthers have only one set of the genetic blue-print (one set of chromosomes), and are haploid.

Irradiation of haploid cells and tissues by anther or ovule culture produces plants in which mutated genes show up immediately. This is because the second set of genetic blueprint which may hide such mutations is absent in the haploids. The selected haploid plants then can be made to produce dihaploids with the same chromosome number as is in the normal diploid plants.

The production of haploid mutants and the subsequent production of dihaploids with mutated genes is a fast and rapid method to produce improved varieties in seed propagated crops, such as barley, rice, rape seed, maize, and wheat. The availability of cheap and efficient methods for haploid production from

the local cultivars of grain and legume crops in developing countries is thus essential to utilize mutation based breeding methods.

■ **Somatic embryos.** Yet another *in vitro* method is capable of producing embryos without sexual crossing of plants. Such embryos, called somatic embryos, can be produced from callus and cell suspension cultures, and sometimes directly on the surface of the leaves. Somatic embryos can be routinely produced in many plants, e.g. carrot, cauliflower, citrus, date palm, potato, sweet potato. The somatic embryos in many cases originate from single cells or only a few cells. Irradiation of such cells leads to the production of plants which produce stable and solid mutants. This eliminates the need of repeated culture of tissues to separate mutated from non-mutated cell sectors.

MULTIPLE APPLICATIONS

The improvement of many trees — among which are many important fruits such as date palm, apple, plum, cherry, orange, citrus — through conventional breeding and their propagation is a long and slow process. Induced mutagenesis in combination with micropropagation can be used for producing short height, fast-growing types. This is even more important for short rotation forestry intended for biomass production for fuel wood and paper pulp.

Plant and cell tissue culture techniques allow mutagenesis of large populations of cells and miniature plants in a small space. Thus, it is possible to

grow millions of cells in a petri-dish or in a flask, and irradiate and multiply them on defined media to regenerate plants.

An important component of any plant breeding programme is the selection of desired genotypes. The availability of efficient and rapid methods of selection is essential to screen large populations of plants. There is increasing evidence that for some traits, *in-vitro* grown cells, somatic embryos, and miniature-sized plants can be subjected to selection for disease resistance, tolerance to salinity, heat, cold and freezing. The *in vitro* selected plants can then be tested for their performance in the field. Thus, *in vitro* mutagenesis and *in-vitro* propagation can be integrated into the conventional selection to speed up breeding of vegetatively propagated plants. *In vitro* culture is also important to conserve local, well-adapted clones which are upgraded through mutation induction. It also is a useful technique, followed by irradiation and micropropagation, for embryos of hybrids where seed set is extremely low. Overall, *in vitro* applications widen the genetic variability and promote exchanges of genetic material in the hybrids of distant parents by recombining pieces of the parental chromosomes.

Tissue culture often is mutagenic by itself; plants which originate from cell and tissue culture occasionally do not resemble the donor plant. Such tissue culture induced variation, called somaclonal variation, can also be combined with mutation induction as an additional source of widening the gene pool.

Selected mutants can be multiplied in large numbers and rapidly in a disease-free condition through micropropagation. The technology of micropropagation is so advanced that now there are over 200 commercial companies in North America and more than 100 in Europe producing millions of plants through tissue culture. *In vitro* cultured plants can also be shipped after irradiation to countries which do not have their own radiation facilities.

The technology of plant tissue culture has developed at a very fast rate. It is being used to multiply high quality planting material at commercial scale in many countries in Europe and North America, but only a few countries in Asia and Africa.

In many developing countries, this technology either does not exist or is just in the stage of infancy. Since a major cost component in plant micropropagation is labour, developing countries can gainfully make use of these techniques, and at the same time create additional employment by establishing low-cost but high-tech based industry for plant and crop multiplication. In some African countries, the only existing laboratories for plant tissue culture in relation to mutation techniques have been established with the help of the IAEA. These laboratories could be expanded and used for training and capacity building of these countries in micropropagation and production of disease-free plants. □