quickening the pulse of nature

Mutations occur spontaneously in all living things. By changing the chemistry of the organism's genetic material or altering the structure of a chromosome a mutation changes the structure or function of the organism and its offspring. Most often the mutation is harmful, and the change is erased by natural selection. Once in a while a mutation is beneficial, increasing the organism's chance to survive and reproduce or, in the case of plants and animals that are directly useful to man, increasing its value. In this article Björn Sigurbjörnsson, Deputy Director of the FAO/IAEA Joint Division of Atomic Energy in Food and Agriculture, explains the work of the "mutation breeder" and the rôle of nuclear techniques in helping to bring about "the Green Revolution".

This article is based on a paper by Dr. Sigurbjörnsson published in full in the "Scientific American" earlier this year, and on other material.

The principal mechanism for the induction of mutations with which we are concerned here is the irradiation of seeds, although mutations may also be induced by irradiation of entire plants and by treating seeds with mutagenic chemicals. Artificial induction of mutations was first discovered in 1927, but the first attempts at inducing mutations to improve plants were not successful and the idea fell into disfavour. In recent years, however, the state of knowledge has improved to the point where it is possible to obtain generally good results. More than 100 varieties of induced-mutant crop plants — including wheat, rice, barley, oats, soybeans and a number of other legumes, fruit trees and ornamental plants — have been released to growers and are being raised by farmers on millions of acres throughout the world. Some of these varieties have played a part in the "Green Revolution", which has resulted in substantial improvements in crop yields, and further improvements in crop plants through induction of mutations promise to play a significant rôle in sustaining and consolidating this revolution.

In 1964, the IAEA and FAO combined their activities by founding the Joint Division of Atomic Energy in Food and Agriculture. Since then, the Joint Division has conducted several co-ordinated programmes on mutation plant breeding for crop improvement. One of the first of these, a research programme on the use of mutations induced in rice, was implemented mainly by institutes in South East Asia, and led to the development of several new strains. A co-ordinated research programme now under way is concerned with the improvement of the protein content of cereals and legumes. Participating scientists have already reported encouraging results in improving the protein content of rice and barley and in improving high-protein crops such as beans and peas. Rice mutants with significantly higher protein content have been produced in Japan and, in Pakistan, protein content has been raised in "miracle rice". In Japan, radiation treatment of one variety of rice resulted in a series of mutants with a range of protein content duplicating the entire range found in nature.

The alteration of heredity

In essence, mutations are sudden changes in the hereditary material of an organism, including all such changes that cannot be accounted for by the normal recombination of the units of heredity. Being the very source of genetic variability, mutations are ultimately responsible for the evolution of all present forms of life. (A related thought: the eminent Swedish plant breeder Professor Åke Gustafsson stressed recently that the Green Revolution was only a beginning. What we are really concerned with is a "Green Evolution", maintained by agricultural research and technology and accompanied by economic, social and political adjustments.)

Natural or spontaneous mutations have a number of causes. Cosmic rays, which bombard the earth constantly, can penetrate matter readily. If one strikes a chromosome it can produce a mutational change. Normal physical phenomena such as heat can cause mutations; so can pure oxygen under pressure. It has been shown that genetic material can mutate simply through the process of aging. A number of substances including caffeine have been shown to be potentially mutagenic.

But the rate of spontaneous mutations is rather low. Among 10 000 barley seedlings one can expect to find one or two that are not the normal green, having undergone spontaneous mutations of the sort which affect their chlorophyll. The rate of spontaneous mutation is also variable, some genes mutating more often than others.

Pioneering work in the induction of mutations was done by the American geneticist H. J. Muller, who published in 1927 a paper describing his discovery that mutation frequency is increased following irradiation of the fruit fly *Drosophila melanogaster* with X-rays. He later received the Nobel prize for this work. At almost the same time the induction of mutations was demonstrated in corn and barley by another American worker, L. J. Stadler. Even at that early stage Muller thought that induced mutations had the potential to revolutionize plant breeding.

Natural evolution is based on three main factors: spontaneous mutations; hybridization, which reshuffles mutations into almost unlimited genetic patterns; and natural selection, which favours the perpetuation of individuals with genetic patterns which make them better able to survive and to reproduce. Traditionally, the plant breeder has been able to control two of these factors only — hybridization, by making deliberate choices of parents when crossbreeding plants, and natural selection, by applying deliberate pressures and by careful selection of superior plants. When the possibility of artificial induction of mutations was demonstrated many plant breeders undertook to try their hand at this third cornerstone of evolution.

But in the early days little was known about the sensitivity of seeds to radiation, the effect of the seed's physiological condition at the time of treatment, the effect of radiation on the plant or how succeeding generations would develop. Even when successful mutations were achieved, their application was hampered by insufficient understanding of the need to establish specific breeding objectives, choose appropriate parents and handle properly the succeeding generations. As a result, the expected "miracles" failed to materialize.

A few plant breeders, however — among them Prof. Gustafsson, of the University of Lund —, realized that the fault lay not with the principle of induced mutations but with these other factors. Their work helped the pendulum to swing back.

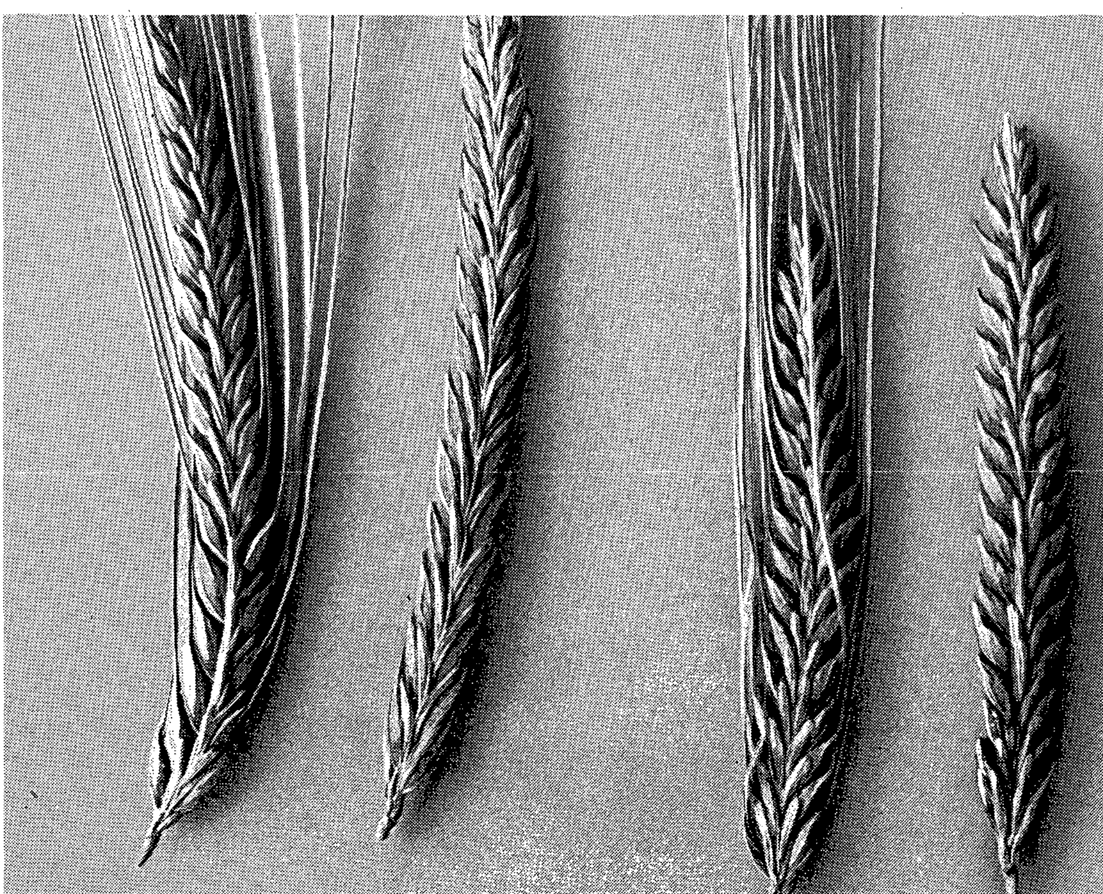
Principles and practice

Until fairly recently X-rays and gamma rays were the principal agents employed to induce mutations. They were not always effective, because the importance of controlling the physiological conditions of the seed — primarily its content of oxygen and moisture — at the time of treatment was not adequately appreciated. On the other hand, treatment with neutrons has entailed the difficulty of measuring dosages reliably in nuclear reactors, particularly when fast, or highly energetic, neutrons were employed.

On the left, normal barley; on the right, a mutant induced by irradiation with fast neutrons. The shorter, denser spike is clearly apparent. For greater clarity the awns of the right-hand spikes of both control and mutant have been removed.

The difficulties with the effective use of these two types of radiation have recently been overcome through intensive studies by teams of biologists, chemists and physicists, resulting in vastly improved efficiency in inducing mutations. Ionizing radiations are now readily available to most plant breeders — X-rays from X-ray tubes and other devices, gamma rays from radioactive isotopes (chiefly cobalt 60 and cesium 137), and neutrons from reactors or special neutron generators. It must be said, however, that considerable room for improvement remains.

In addition to the ionizing radiations as a means of inducing mutations there are a number of chemical mutagens with effects so similar to that of radiation that they have been called radiomimetic substances. The most effective are several derivatives of sulfonic acid, notably ethyl methane sulfonate (EMS). Chemical mutagens are ineffective when applied to the vegetative parts of a plant, and this is a serious limitation on their use. As a result they are used still only as a supplement to the use of radiation in treating seeds. In practical plant breeding it is common to rely on more than one mutagen to ensure the induction of a maximum number of mutations. Many plant breeders make a practice of having different batches of seed treated with gamma rays, neutrons and one chemical mutagen, such as EMS. They then choose from the material resulting from these three treatments. Combined treatment, using different mutagens for the same seeds, has not proved to be advantageous.



Induced mutations can be used in plant breeding in many ways. The most direct way is to multiply the seed of the induced mutant and to make it available to growers as soon as enough seed has been produced. An advantage of this method is that an improved variety can be developed in a comparatively short time.

The direct use of induced mutants has the second advantage that the improvement usually achieved is highly specific. An ideal application of this principle can result in the restoration of a generally successful and adapted variety that is on the decline for want of a specific agronomic trait such as strength of stem or resistance to a disease. The alternative would be to find the missing trait in another variety, cross-pollinate that variety with the commercial one and then go through a long selection procedure, selecting for all the good attributes of the original variety plus the one trait sought from the other variety. This result is not always easy to achieve, since the desired trait may be governed by a gene located on the same chromosome as other genes that give rise to undesirable characteristics. Such linked genes are often difficult to separate by conventional means. Even here a mutagenic treatment is useful, because it can result in breaking up such linkages — that is, freeing the desired gene from its undesirable associates.

Mutagenic treatment of the commercial variety may, of course, result in a mutation giving rise to the desired trait directly, without resort to a cross-pollination programme. It is quite common for a plant containing such an induced mutation to remain unchanged in most other significant agronomic traits, although there are exceptions.

Restoring quality to pasta

This special attribute of induced mutations is extremely important and provides the plant breeder with a unique complementary approach. A number of commercial varieties have been developed on this principle. New varieties of Italian durum wheat provide a good example. The Italian pasta does not really live up to its reputation unless one uses special kinds of durum wheat. The Italian standard is the old variety called Cappelli, which was considered a high-yielding variety. When yields of bread wheat were increased by the application of large quantities of fertilizer and by the introduction of new varieties of wheat that responded to the fertilizer, however, the old Italian pasta varieties lagged behind. The problem is that when more fertilizer is applied the old wheat varieties, in contrast to the new dwarf types, respond by growing in height. The stems become weak and, particularly during rain and wind, are not able to carry the weight of the grain heads. The result is that the plants lodge — that is, their stems bend or break, resulting in a reduction in yield rather than an increase. Improvement was achieved by crossing the old wheat with other high-yield and lodging-resistant varieties, but the quality of the pasta suffered.

G. T. Scarascia-Mugnozza, who was then working at the Italian nuclear research centre at Casaccia, solved the problem by treating seeds of Cappelli with neutrons then selecting for shorter and stronger stems and high yield while retaining the other good qualities of Cappelli. The two new mutant varieties, named Castelfusano and Castelporziano, are considerably shorter than Cappelli, and their stem is much stronger, making them essentially immune to lodging. Their yield is higher, and their grain quality remains nearly the same as that of Cappelli for one of the mutants; the other has a quality that is lower but still acceptable.

Is it really worthwhile?

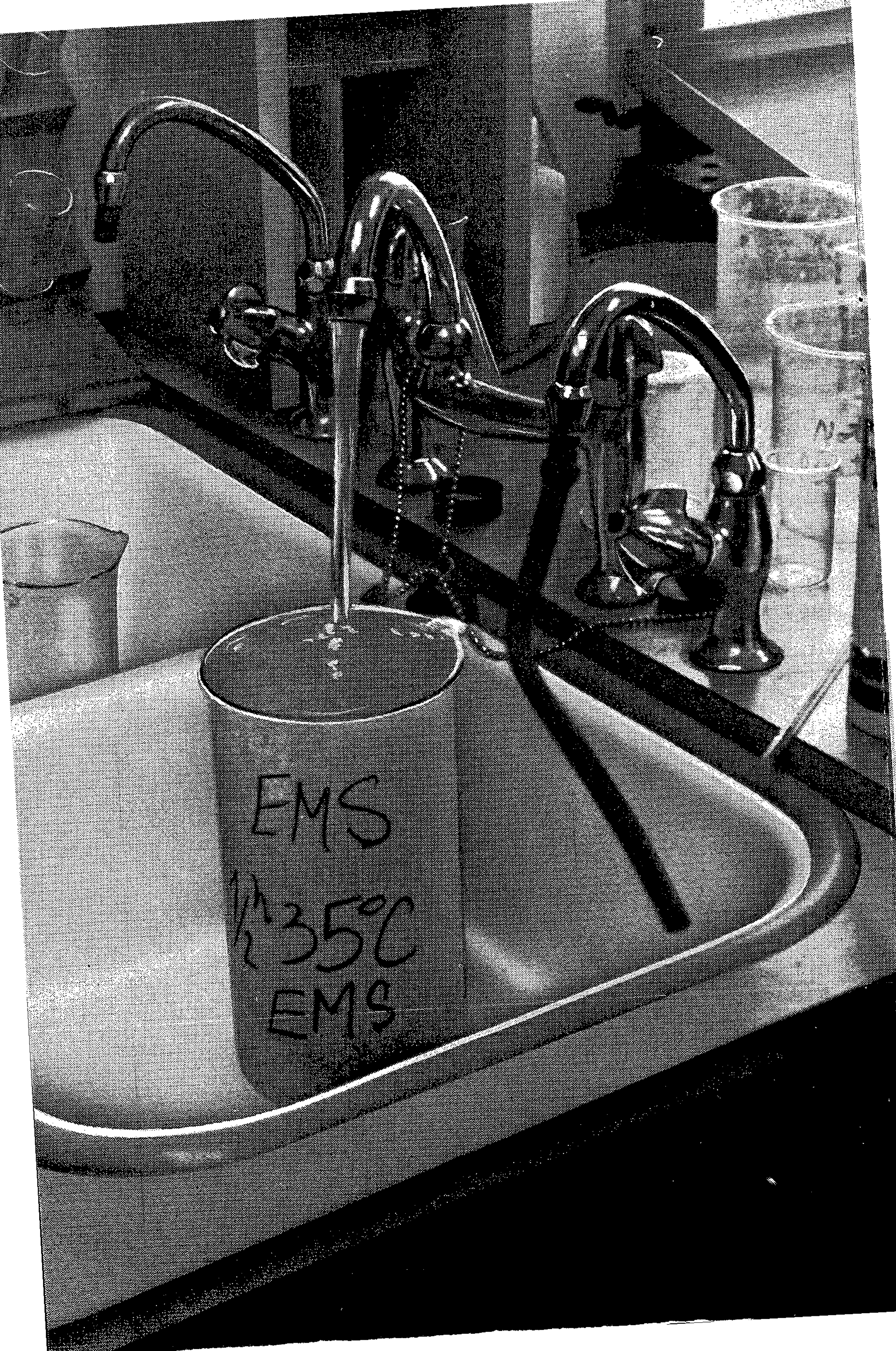
The attitude among plant breeders toward the possibilities offered by radiation-induced mutations ranges from over-enthusiasm to scepticism. Our Joint Division of the FAO and IAEA has attempted to steer a middle course between these views. On the basis of our own experiments and advice from prominent breeders we have prepared a *Manual on Mutation Breeding*, which was published in 1970. Our laboratory at Seibersdorf, near Vienna, and the Austrian Atomic Energy Research Centre have collaborated in developing a facility for use in nuclear reactors, where seeds can be irradiated with neutrons. The facility screens out gamma rays and slow neutrons so that the dosage of fast neutrons can be determined with precision.

We have organized coordinated research programmes in a number of countries. As a result of a typical programme under way in Southeast Asia several improved mutant lines of rice are undergoing advanced agronomic testing. Korean and Japanese agronomists working in collaboration have isolated mutants resistant to the devastating disease called blast. An Indian group has improved the cooking quality of rice by conferring the fine grain characteristics of the 'indica' type of rice plant, which is non-sticky and rich in the starch amylose, to the 'japonica' type, while retaining all the other traits, including resistance to bacterial blight, of the japonica strain. This is a particularly significant achievement in view of the difficulty of bringing the combination about by crossing the two types, which had been tried with limited success for 20 years.

In co-operation with other interested agencies our Division has organized international field trials of promising mutants of rice and wheat. Rice mutants that had been developed before the coordinated programme started were tested immediately, in collaboration with the International Rice Research Institute. The indica mutants did not yield quite as well as the new high-yield varieties from the Philippines, although the eating quality and local adaptability of some of them better suited the countries where they were developed. The japonica mutant Reimei was the highest-yielding line tested in its region of adaptation. We are now planning international trials to test the mutant lines that have been developed under our programme.

The wheat trials involved the durum mutants developed in Italy by Scarascia-Mugnozza. These trials have been carried out every year since 1966 in a number of countries around the Mediterranean and in the Middle East. The mutants have shown superiority in yield and lodging resistance in nearly all the countries over those years when compared with the best local durum varieties and with other control varieties.

Our Division is now considering coordination of research on the use of induced mutations to improve specific deficient characteristics of crop plants. Particular attention is being given to problems of disease resistance and protein content; an agreement between the IAEA and the Gesellschaft für Strahlen- und Umweltforschung, Munich, on participation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by the Gesellschaft in a worldwide research programme aimed at improvement of protein content and quality of crops which are especially important to developing countries was signed recently. A great deal of practical research for this programme is to be carried out in the developing countries themselves, in addition to work in the Federal Republic of Germany and at the Agency's own laboratories. We plan also to coordinate studies on the application of radiation and isotopes to analytical techniques for rapid mass screening of plant seeds for improved protein. If successful, these ventures could contribute significantly to the problem of providing adequate supplies of nutritious food for the world's growing population.

A stage in the induction of mutations in barley: the seeds (in the beaker), having been treated with the chemical mutagen EMS, are washed thoroughly. Photo: IAEA/Arzensek