THE DESIGN AND USE OF A Co^{60} IRRADIATION UNIT IN THE UNITED STATES EXHIBIT, WORLD AGRICULTURAL FAIR

J. L. BREWBAKER AND M. S. SWAMINATHAN

Brookhaven National Laboratory, New York, and Indian Agricultural Research Institute, New Delhi

A COBALT-60 irradiation facility was installed and operated in the United States Pavilion at the World Agricultural Fair in New Delhi from December 11, 1959 to March 1, 1960. The unit was designed for the gamma irradiation of biological and other research materials as well as for demonstrational purposes, and operated as a service unit to scientists throughout the Fair. The following description of the unit and discussion of its uses and limitations in agricultural research is made in view of the widespread interest and use which the unit evoked from Indian scientists.

Design of the Facility

The cobalt-60 facility was designed, fabricated and installed under the direction of Mr. Otto Kuhl of the Nuclear Engineering Division of Brookhaven National Laboratory, under contract for the U.S. Atomic Energy Commission. The total strength of the radioactive Co^{60} source was 4,750 curies, distributed more or less evenly among nine steel-encased cobalt plates. The plates were placed in radiation chambers at the bottom of a pool of filtered water, 10 feet deep, ensuring that no appreciable radiation (0·3 mr./hr.) occurred at the water-surface. The pool was surrounded by glass panels, and materials to be irradiated were lowered into the pool from a platform 3 metres above water-level. The nine cobalt plates employed in the facility measured 2½" × 13" and about ⅛" in thickness. Each of these plates had been exposed for nearly two years to the neutron flux in Brookhaven's pile-type reactor and contained approximately 500 curies of Co^{60}. The plates had a specific activity of about 2 curies per gram.

Four cylindrical irradiation chambers were designed to accommodate the cobalt plates, and were spaced one metre apart at the bottom of the pool. The plates were arranged vertically in the walls of the irradiation chambers. Materials to be irradiated were placed in water-tight steel containers which could be guided neatly into the hollow core of the chambers. The containers were lowered by winch, two of which were operated by automatic power units. The outer diameters of the 4 containers were 2¼" for containers 1 and 4, 4" for container 2, and 3" for container 3. The four chambers were operated throughout most of the Fair with 1, 2, 4 and 2 cobalt plates in chamber 1, 2, 3 and 4 respectively.

The ferrous sulphate oxidation method[1] was used to calculate the gamma radiation dosages. The spectrophotometric measurements were carried out within an hour of irradiation at the Division of Mycology and Plant Pathology of the Indian Agricultural Research Institute. On the 1st of January 1960, the calculated dose rates were 30 150 rad./hr. in chamber 1, 60 230 rad./hr. in chamber 2, 185 150 rad./hr. in chamber 3, and 102 850 rad./hr. in chamber 4. These were readjusted down by one per cent. monthly thereafter to compensate for decay. The dose rate dropped by as much as 30% from the centre to the periphery of the containers; hence samples were irradiated in the central core of the chambers insofar as possible.

Use of the Facility

The gamma irradiation facility proved to be a very popular exhibit with the general visitors, owing to the blue Cerenkov glow seen in the water when the lights were switched off. The great potentialities offered by this excellent research tool also attracted the interest of numerous scientists and scientific institutions in India; as a result, the irradiation chambers were loaded with material received from all over the country throughout the duration of the Fair. This made the exhibit a very unique and distinctive one and it will take several years for the investigators who had material irradiated to analyse fully the effects induced by the dosage given.

A total of 4,757 samples submitted by 94 research workers were irradiated during the 80 operating days of the Fair. About 160 types of research materials were handled, including (1) seeds of 111 different species, (2) roots, tubers and cuttings of 20 species, (3) pollen and embryos of 11 species, (4) 10 species of micro-organisms, and (5) miscellaneous items such as culture media, fruits, fern spores, chemical solutions, glass slides, cylinders and white rats. In view of the great diversity of the material treated, the dosages given varied widely, ranging

from 200 rads to 3 million rads. The item "rads" refers to the gamma dosage as calculated by the chemical indicator techniques. In general practice this may be considered equivalent to the more familiar unit, "r" or roentgen. Most investigators left to the discretion of the operators the dosages to be administered. Wherever the approximate LD-50 (50% lethal dose) was already known, the material was treated with several dosages, keeping the LD-50 dose as the modal class. For seeds of cereals like wheat and barley the LD-50 dosage lies between 15,000 and 30,000 rads. On the other hand, for plants like mustard and linseed, the LD-50 dosage is over 100,000 rads. For seed material, the treatments thus ranged from 1,000 to 300,000 rads. Cultures of *Penicillium*, *Streptomyces* and other micro-organisms were given dosages about a modal class of 25,000 rads. This modal dosage was 3,000 rads for most tubers and cuttings. For material such as seeds of water chestnut (*Trapa nutans*) and cuttings of tapioca (*Manihot utilissima*), for which no previous data existed, a broad range of treatments was given. A dose of 800 rads was delivered to rats; for treating them, a special container equipped with air hoses was lowered to a point on the floor of the pool where the dose rate was approximately 2,400 rads per hour. Special chambers were also designed to treat seeds in a pure oxygen atmosphere. Thus, the set-up of the source offered sufficient scope to undertake critical experiments on a wide range of radiobiological problems.

Induced Mutations and Plant Breeding

Over 90% of the material irradiated at the Co^{60} unit came from plant breeders. The primary interest in these cases was that of the induction of mutations of economic value. In view of the widespread interest of plant breeders in India in the technique of mutation breeding, a few general comments here may not be out of place.

Firstly, huge quantities of seed material (several pounds of paddy seeds, many ounces of tobacco seed, etc.) were often sent for irradiation. While it is presumed that these seeds may be derived from essentially homozygous lines and that suitable controls might have been kept by the investigator, it was apparent that some of the plant breeders who sent samples expected to isolate mutations in the first generation following treatment. While some genetically-controlled phenotypic changes, resulting largely from the deletion of epistatic genes, may be manifested in the year of treatment (particularly in polyploid plants),[2] it is essential that the second and further generations of the irradiated material should be grown to detect the recessive mutations which constitute a vast majority of induced mutations.

Secondly, recent results[3,4] have emphasized that following irradiation, even a self-fertilised plant is cross-pollinated to a great extent owing to varying degrees of radiation-induced pollen sterility in the X_1 plants (plants grown from irradiated seed). It thus becomes necessary in critical experiments to make controlled self-pollinations of each X_1 plant and to grow its progeny separately during the next season. Unless such care is taken, segregation in X_2 lines cannot be attributed to mutation with any degree of certainty. Experiments carried out at the I.A.R.I. have shown that it would be preferable in plants like wheat and paddy to harvest and sow the seeds from every ear of X_2 plants separately. Thus, a plant breeder who has 200 to 300 X_1 plants will have a very large material to handle and study during the X_2 generation. It is now well established that success in mutation breeding will depend on the size of the X_2 and subsequent populations and the efficiency of the screening procedures adopted. An intimate knowledge of the cyto-genetic make-up of the plant is also highly desirable. Taking to mutation research as a part-time activity may hence lead the research worker nowhere both from the applied and fundamental points of view.

Thirdly, some research workers interested in pollen irradiation sent samples of pollen from plants like wheat. An essential prerequisite in such work is information concerning the duration for which pollen remains viable. As a rule, trinucleate pollens lose their viability a few hours after anthesis while in plants in which the pollen is binucleate at the time of anthesis, the viability extends over several days and even months.[5] The pollen grains of wheat and paddy are trinucleate and hence the duration for which they remain viable is very short (a few hours at the maximum). Sending pollen of such plants over long distances for irradiation and later using them for pollination will hence be a futile process.

Fourthly, a considerable number of seed samples submitted for irradiation represented highly cross-pollinated species. Although the majority of these were for use in botanical studies or research seeking unique mutant types, some were irradiated as an adjunct to plant breeding programmes with the general objective of

increasing yields. Not only is the distinction of induced mutations from natural variability impractical if not impossible in such material, but it is the conviction of most plant breeders that irradiation should be held in reserve as a source of new variability in such species until conventional breeding practices have been most thoroughly surveyed. This is particularly important in the improvement of polygenic characters, for which gene mutations of the typical recessive monogenic nature afford little promise of breeding progress. It is important as well to note that many induced mutations result from cytological aberrations which otherwise affect the plant adversely, *e.g.*, reducing fertility and seed production.

We would like to take this opportunity to caution research scientists and administrators against the growing tendency to look upon radiation as a magic tool in plant improvement. Few countries, developed or underdeveloped, have adequately exploited in their plant breeding programmes the variability already present in the indigenous material or in material that could be introduced easily from other parts of the world. It is important therefore that plans for the use of radiation in plant breeding should not be at the expense of finance and trained personnel needed for carrying out an effective breeding programme conducted on a conventional basis. Until we know more about the experimental control of the frequencies and types of induced mutations, radiation should be considered only as a spcial research tool, valuable particularly in causing specific deletions or translocations and breaking tight linkage groups.

Geneticists who have reported promising results with this tool are unanimous in holding the view that plant breeders should regard this method as *supplemental* and not *substitutional*. It takes nearly as many years to convert an induced mutation into a finished product suitable for release for cultivation as it takes for breeding a new variety.

Trends in Gamma Ray Installations

Several hundred gamma irradiation units are presently employed throughout the world in biological, medical and agricultural research. The radioactive isotope of cobalt, Co^{60}, has been used in most of these units. The most popular types of cobalt facilities have been (1) gamma rooms, the cobalt commonly attached to the lid of a lead container which can be raised by remote control for irradiations, (2) gamma pools, such as the one described here, and (3) gamma fields, for the purpose of providing continuous irradiation to living organisms (two such units occur in India, at the IARI and Bose Research Institute). The primary advantage of the pool-type unit is that extremely "hot" sources may be employed with great safety and minimal expense. For example, the 4,750 curie source exhibited in the pool at Delhi would require a field at least one mile in diameter, or a room provided with 50-inch thick concrete walls for reasonable safety.

Owing to its relatively short half-life of 5·3 years, Co^{60} loses about 1% of its energy monthly and needs to be recharged in a reactor fairly often. Hence other gamma emitters like $Cesium^{137}$ are presently gaining in popularity. Cesium is a fission product with a half-life of 30 years obtained from used fuel elements of a nuclear reactor and has only recently become available at a cost comparable with that of cobalt. While Cs^{137} emits a single photon of energy 0·661 Mev, Co^{60} emits two photons of gamma energy 1·17 and 1·33 Mev. As a result, a curie of Co^{60} produces a field of 1·35 r./hr. at one metre in contrast to 0·356 r./hr. given by a curie of Cs^{137}. In general the lower energy gamma rays of cesium are slightly more effective in producing biological changes such as mutations, but are considerably less penetrating. The lower energy and penetrability of Cs^{137} make it impractical for use in medical therapeutic units. However, Cs^{137} is expected to be very useful for the irradiation of biological materials in pool-type units as the one described here, in gamma rooms (as installed at the National Institute of Genetics, Mishima, Japan) or in a gamma field (as under design in Germany and Spain). Data gained from these and other Cs^{137} pilot units will help to evaluate the suitability of Cs^{137} as a partial replacement for Co^{60} in biological research.

Acknowledgements

The first author (J. L. B.) is indebted to the Brookhaven National Laboratory and the U.S. Atomic Energy Commission for deputing him to act as Director of the Co^{60} irradiation unit in the American Pavilion. The second author (M. S. S.) is grateful to Dr. M. S. Randhawa, Dr. B. P. Pal and the Ministry of Food and Agriculture for nominating him as the Scientific Liaison Officer with the U.S. Atomic Energy Commission. Our sincere thanks are due especially to Mr. Otto Kuhl of Brookhaven National Laboratory, who was largely responsible for designing and setting up the source, for all his advice and assistance. Dr. S. Majumder and Messrs. C. Bhatia, J. Prasad and M. Upadhya