27. PROCEDURE FOR MUTATION BREEDING

Treating a biological material with a mutagen in order to induce mutations is known as mutagenesis. Exposure of a biological material to a radiation like X rays,_gamma - rays, etc. is known as irradiation. When mutations are induced for crop improvement, the entire operation of the induction and isolation, etc. of mutants is termed as mutation breeding. A mutation breeding programme should be clearly planned and should be large enough with sufficient facilities to permit an effective screening of large populations. The various steps involved in mutation breeding are briefly discussed below.

Objectives of the Programme

A mutation breeding programme should have well defined and clear-cut objectives. **If** the experimenter starts a mutagenesis programme just with the hope that he will discover something useful, he is most likely wasting his time and resources. This is because the ratio of beneficial to useless mutations is very small (1 in 800 mutations, that is, about 0.1 % of the mutations), and identifying desirable mutations from among the undesirable ones is a very difficult task indeed. Further, if a character governed by oligogenes is to be improved, the procedure for the handling of treated populations would be different from that when a polygenic trait is the target for improvement.

Selection of the Variety for Mutagen Treatment

Generally, the variety selected for mutagenesis should be the best variety available in the crop. This is particularly so when polygenic traits are to be improved. It serves no purpose to isolate desirable mutants in a less adapted inferior variety only to discover that the mutant lines have no agricultural worth, or that the mutants have to be used in a hybridization programme for transferring the mutant characteristics to a superior variety. In certain situations; however, it may be desirable to isolate mutants in varieties other than the best one. For example, an extensive search is being made for alternative dwarfing genes in cereals, particularly in wheat and rice (*O. sativa*). In this situation, dwarf and semi dwarf mutants would have to be isolated from tall varieties, which obviously would not be the best varieties of these crops.

Part of the Plant to be Treated

Seeds, pollen grains or vegetative propagules (buds and cuttings) or even complete plants may be used for mutagenesis. Which plant part should be used for mutagen treatment depends primarily on whether the crop is sexually or asexually propagated and on the mutagen to be used.

In sexually propagated crops, seeds are the most commonly used plant part. Dry dormant seeds are biologically almost inert and they can stand a range of extreme environmental conditions, such as, soaking, desiccation, heating, freezing, oxic or anoxic regimes, etc. Mutagenic treatment of seeds is essentially a treatment of embryo meristems .

Since mutation is a single cell event, the M1 plants will carry an induced mutation only in parts of the shoot, i.e., they will be chimaeras. Pollen grains may be used, but they are infrequently used because (1) it is difficult to collect large quantities of pollen grains in most crop species, (2) hand pollination (with treated pollen) is difficult, and (3) pollen al is relatively short. Pollen grains are the only plant part, which can be successfully treated with UV radiation. A pollen monolayer is exposed to UV rays of 250 to 290 nm; of the biological effects induced by UV rays are almost comparable to those produced sparsely ionizing radiation. In case of clonal crops, buds or cuttings are used for mutagenesis.

Radiation (except UV) is suitable for use with all the three plant parts and even with the plants. Whole plants are generally irradiated during the flowering stage so that it is equivalent to the irradiation of pollen grains and egg cells. However the treatment of whole requires special facilities (a gamma garden) and is possible in a few places only. Chemical mutagens are best used with seeds, but some workers have used them with vegetative propagules as well.

Dose of the Mutagen

The usefulness of a mutagen and the type of treatment required to obtain a high efficiency pendent upon specific properties of the mutagenic agent employed (its effectiveness, effect relationship and mode of application) as well as on specific characteristics of the biological system to be treated (the sensitivity of the treated tissues depending upon anatomical, physiological, biochemical and genetic peculiarities). The most appropriate plant or stage to be treated requires a thorough knowledge of the organisms and a clear definition of experimental objectives.

Mutagen treatments reduce germination, growth rate, vigour and fertility (pollen as well lie). There is considerable killing of plants during the various stages of development after mutagen treatment; thus survival is reduced considerably. Mutagens generally induce a high frequency of chromosomal changes and mitotic and meiotic irregularities. Usually, the damage increases with the mutagen dose, but it may not necessarily be proportional. An optimum dose is the one, which produces the maximum frequency of mutations and causes the minimum killing.

The dose required for high mutagenic efficiency depends on the properties of the mutagenic agent, of the solvent medium and of the biological system. Many workers that a dose close to LDso should be the optimum. LDso is that dose of a mutagen, which would kill 50 per cent of the treated individuals. LDso varies with the crop species and with mutagen used. A preliminary experiment is generally conducted to determine the suitable mutagen dose. In general, an overdose is likely to kill too many treated individuals, while an underdose would produce too few mutations.

Dose of the mutagen may be varied by varying the intensity or the treatment duration. In case of radiation, intensity may be varied by changing the radiation source or by changing the nee from the radiation source of the material being irradiated. Intensity in the case of chemical mutagens may be varied by changing the concentration of mutagens.

Giving Mutagen Treatment

The selected plant part is exposed to the desired mutagen dose. In case of irradiation, the plant parts are immediately planted to raise M1 plants from them (pollen grains are used for pollination). In case of chemical mutagens, seeds are usually presoaked for a few hours to initiate metabolic activities, exposed to the desired mutagen and then washed in running tap water to remove the mutagen present in them. The treated seeds are, usually, immediately planted in the field to raise the M1 generation. M1 is the generation produced directly from the mutagen-treated plant parts without a recourse to sexual or asexual reproduction. But when pollen grains are treated, the generation resulting from the seeds that were produced by pollination with the treated pollen grains would be the M1 generation. M2, M3,M4, etc. are the subsequent generations derived from M1, M2, M3, etc. plants through selfing or clonal propagation.

Handling of the Mutagen-Treated Population

Treatment of seeds and vegetative propagules commonly produces chimaeras. A chimera is an individual with one genotype in some of its parts and another genotype in the others. Shoot-tip meristem usually has three functional layers as follows: (1) L1 gives rise to epidermis, (2) L2 produces a part of leaf mesophyll and gametes, and (3) L3 yields the rest of plant body. When the whole of Ll, L2 or L3 layer is affected, the chimaera is known as periclimal chimaera, while in a sectorial chimaera only a part of Ll, L2 or L3 layer is affected.

In sexually reproducing species, only the L2 chimaera (periclinal or sectorial) will be transmitted to the next generation; other chimaeras will not be recovered since these layers do not contribute to the production of gametes. In clonal crops, however, all chimaeras can be utilized either as periclinal chimaeras or by producing homogeneous individuals through sexual reproduction (only if the L2 layer is affected), tissue culture or certain other horticultural manipulations, e.g., wounding, etc., which induce production of adventitious shoot buds (all chimaeras are utilized). Sectorial chimaeras are unstable in clonal crops and have to be made periclinal through successive clonal propagation and selection for stability.

Mutations usually occur in small sectors of the meristem and, as a result, only a part of the plant is affected. One or more sexual or clonal generations coupled with selection are necessary to obtain a stable mutant phenotype. Mutant alleles are generally recessive, but some dominant mutations may also occur. In case of sexually reproducing crops, mutation breeding utilizes both recessive and dominant mutations and, in addition, excellent opportunities exist for mutation breeding for polygenic traits. Mutation breeding in clonal crops, however, primarily depends on dominant mutations; recessive mutations may also he utilized provided the clone used for mutagen treatment was heterozygous for the gene in question. For example, if recessive mutant allele a is to be useful in a clonal crop, the clone used for mutagenesis has to have the genotype Aa. Such situations are, however, rare; more frequently, the mutants useful in the improvement of clonal crops contain dominant mutations, and they may even include changes in chromosome structure or even number.

Mutations are called macro or micro-mutations depending on the magnitude of phenotypic effect produced by them. A macromutation produces a large phenotypic effect recognizable on individual plant basis; obviously, such mutations are oligogenic in nature and can be easily selected in the M2 generation. In contrast, a micromutation has a small phenotypic effect that cannot be recognised on individual plant basis; it can be detected only in a group of plants and often statistical treatment of data may be necessary. Obviously, macromutations are polygenic in nature and selection for them is delayed till M3 or a later generation.

The detailed procedure for handling of M2, M3, etc. generations will differ depending mainly on the oligogenic or polygenic trait to be improved and on the mode of reproduction crop species. The following discussion is based on sexually reproducing species, more particularly, self-pollinated species. Since dominant mutations are able to express themselves in heterozygous

state, mutant plants are selected in M1 and often in M 2 and M3, individual plant progenies are raised and homozygous mutants are selected. Selection for recessive mutations, however, can be taken up in M2 only, but the mutant allele will be homozygous in the M2 itself. Selection for polygenic traits is delayed till M3 generation, and is based on individual plant progenies rather than on individual plants. Generalized schemes handling the mutagen treated populations for oligogenic and polygenic traits are outlined in the next section.