CHAPTER TWENTY

Mutation Breeding

Genetic variability is required to improve a crop for any character. Mutations that occur spontaneously or are artificially induced can be useful sources of variability for the plant breeder.

Mutation breeding has been used to develop improved cultivars of many crops. Sigurbjornsson and Micke (1974) and Sigurbjornsson (1983) reviewed cultivars that had been developed by mutation breeding. The crops and ornamentals they included were bread wheat, durum wheat, barley, oat, rice, soybean, string bean, French bean, navy pea bean, haricot bean, pea, lupine, subterranean clover, seradella, lespedeza, red clover, Italian ryegrass, peppermint, lettuce, tomato, potato, onion, spinach, spring rape, white mustard, castor bean, tobacco, cotton, peanut, peach, cherry, apricot, citrus, apple, carnation, chrysanthemum, dahlia, achimanes, streptocarpus, alstroemeria, rose, and azalea. The characters they cited for which crop cultivars had been improved included yield, lodging resistance, disease resistance, maturity, stem length, food quality, winter hardiness, protein content, shattering resistance, plant type, ease of harvesting, morphology, grain color, seed weight, sprouting resistance, drought resistance, lysine content, and adaptability. It is clear that artificial mutation can be a practical means of obtaining genetic improvement in crop species.

Although cultivars have been developed by mutation breeding, the number is extremely small when compared with the number of those developed by hybridization and selection. Extensive studies of the use of artificial mutagenesis for genetic improvement of crop species during the 1950s and 1960s demonstrated that mutation breeding was of limited value for improving characters of economic value, particularly those that are quantitatively inherited. The relative lack of success of mutation breeding could lead to the conclusion that it has no place in a modern plant breeding program. A more appropriate conclusion, however,

is that the role of mutation breeding for genetic improvement of a crop species should be carefully defined.

It is generally agreed that mutation breeding is most appropriate when a desired character is not available in the germplasm that can be used for hybridization and selection. The frequency of a desired genetic change from artificial mutagenesis generally is low; therefore, the probability of success is greatest when a large number of individuals can be screened for the character.

MUTAGENIC AGENTS

The average rate of spontaneous mutation is approximately 10^{-6} for an individual gene. The goal of artificial mutagenesis is to increase the rate of mutation for the desired characteristic. Mutation rates can be increased by many different means, including temperature, long-term seed storage, tissue culture conditions, radiation, and chemical mutagens. At this time, radiation and chemical mutagens are the most effective. The agent selected depends on its availability, mode of action, and effectiveness for achieving the desired genetic change.

Radiation

Radiation is produced by high energy particles disrupting chemical bonds and transforming one compound into another. Several types of radiation are available (Table 20-1).

X-Rays. X-rays are a commonly used type of radiation for mutagenesis. The treatment can be applied with an X-ray machine capable of producing radiation with the desired wavelength. X-rays with short wavelengths (hard X-rays) have greater penetration but have less potential for creating molecular changes than those with longer wavelengths.

X-rays are electromagnetic radiations created by electrically accelerating electrons in a high vacuum, then decelerating them by having them strike a target, such as molybdenum or tungsten. The abrupt stop of the electron causes the emission of radiation as photons. The photons provide the energy needed to create molecular changes in the cell.

Gamma Rays. Gamma rays are electromagnetic radiations that are produced with the use of radioisotopes and nuclear reactors. Treatments can be given in single doses, or plants can be exposed continuously to gamma radiation over an extended period of time.

The two main sources of gamma rays are cobalt 60 and cesium 137. Because the radioisotopes are dangerous and highly penetrating, they are stored in lead containers and moved by remote control mechanisms to irradiate plant material.

Table 20-1 Properties of Different Types of Radiation Available for Artificial Mutagenesis

Type of Radiation	Source	Description	Hazard	Necessary Shielding	Penetration into Tissue*
X-rays	X-ray machine	Electromagnetic radiation	Dangerous, penetrating	A few millimeters of lead, except for very high-energy installations	A few millimeters to many centimeters
Gamma rays	Radioisotopes and nuclear reactors	Electromagnetic radiation similar to X-rays	Dangerous, very penetrating	Requires very heavy shielding, e.g., centimeters of lead or meters of concrete	Many centimeters
Neutrons (fast, slow, and thermal)	Nuclear reactors (piles), accelerators	Uncharged particle slightly heavier than proton (hydrogen atom), not observable except through its interaction with nuclei in material it traverses	Very hazardous	Thick shielding composed of light elements, such as concrete	Many centimeters
Beta particles, fast electrons, cathode rays	Radioisotopes or accelerators	An electron (+ or −) that ionizes much less densely than alpha particles	May be dangerous	Thick sheet of cardboard	Up to several millimeters
Alpha particles	Radioisotopes	A helium nucleus that ionizes very heavily	Very dangerous internally	Thin sheet of paper	Small fraction of a millimeter
Protons, deuterons†	Nuclear reactors, accelerators	Nucleus of hydrogen	Very hazardous	Many centimeters of water or paraffin	Up to many centimeters

*Penetration depends on many variables, but it is assumed that penetration is into ordinary plant tissue of average density.

†A proton is the nucleus of the common isotope of hydrogen; a deuteron is the nucleus of the heavy isotope of hydrogen.

Source: *Manual on Mutation Breeding*, 1977.

A treatment consisting of a single dose of radiation at a high level is applied in a specially designed facility in a laboratory. For treatment consisting of long-term exposure to gamma rays, plants are grown next to a radioisotope; the distance from the radiation source determines the level of treatment. Specially designed facilities for long-term exposure have been constructed in the field, in the greenhouse, and in controlled-environment chambers in shielded rooms.

Neutrons. The neutrons used for mutagenesis are the product of nuclear fission by uranium 235 in an atomic reactor. In nuclear fission, neutrons with a large amount of energy are produced. Some of the neutrons react with other atoms of uranium to continue the fission process, some are absorbed within the reactor, and some pass from the reactor through outlets. These can be used for mutagenesis. Fast neutrons are those that have high energy levels, as emitted from the reactor. Thermal neutrons containing lower levels of energy are produced by reducing the energy of fast neutrons.

Beta Radiation. Beta electrons are negatively charged particles that are emitted from radioisotopes, such as phosporous 32 and carbon 14. The plant material can be exposed to the radioisotope directly or in solution.

Ultraviolet Radiation. Ultraviolet radiation is used primarily for treating pollen grains because of its low ability to penetrate tissue. Wavelengths from 2500 to 2900 nm are most effective, because nucleic acids have maximum light absorption in that range.

Chemical Mutagens

There is a wide range of chemical mutagens with varied modes of activity. The *Manual on Mutation Breeding* (1977) divides chemical mutagens into seven groups on the basis of the main functional group that determines the type of chemical action (Table 20-2).

Among the large array of available chemical mutagens, the most commonly used are ethyl methanesulfonate (EMS), diethyl sulfate (DES), ethyleneimine (EI), ethyl nitroso urethane (ENV), ethyl nitroso urea (ENH), and methyl nitroso urea (MNH).

TYPES OF MUTATIONS

The four types of mutations that can occur are genome mutations, structural changes in the chromosomes, gene mutations, and extranuclear mutations (*Manual on Mutation Breeding*, 1977). Genome mutations involve changes in the

Table 20-2 Groups of Available Chemical Mutagens

Mutagen Group	Sample Product
Base analogues	5-bromo-uracil, 5-bromo-deoxyuridine, 2-amino-purine
Related compounds	8-ethoxy caffeine, maleic hydrazide
Antibiotics	Azaserine, mitomycin C, streptonigrin, actinomycin D
Alkylating agents	
Sulfur mustards	Ethyl-2-chloroethyl sulfide
Nitrogen mustards	2-chloroethyl-dimethyl amine
Epoxides	Ethylene oxide
Ethyleneimines	Ethyleneimine
Sulfates, sulfonates, sulfones, and lactones	Ethyl methanesulfonate
Diazoalkanes	Diazomethane
Nitroso compounds	N-ethyl-N-nitroso urea
Azide	Sodium azide
Hydroxylamine	Hydroxylamine
Nitrous acid	Nitrous acid
Acridines	Acridine orange

Source: *Manual on Mutation Breeding*, 1977.

chromosome number due to the addition or loss of entire chromosome sets or parts of sets. Structural changes in the chromosome include translocations, inversions, duplications, and deficiencies. (Chaps. 4 and 5).

Mutations that involve a change in a single nucleotide base of a gene commonly are referred to as point mutations. The expression of a gene involves the arrangement of the four nucleotides of DNA in a specific sequence. The expression of the gene can be altered by substituting one nucleotide base for another or by adding or deleting a nucleotide. Gene mutations are commonly associated with use of chemical mutagens, but may occur with radiation treatments. The genetic alteration can cause sterility, can be lethal, or may result in the formation of a new, highly desirable character.

Extranuclear mutations involving components of the cytoplasm are known to occur. Cytoplasmic-genetic male sterility is an example of a cytoplasmic mutation that is useful for crop production. DNA involved in extranuclear mutations occurs in the plastids and mitochondria. Genetic changes in these organelles are transferred from one generation to the next primarily through the egg cells. Ethidium bromide has been used to create cytoplasmic changes in crop species. Additional mutagens that may be considered are acridines, streptomycin, dyes, heavy metal salts, EMS, and radiation treatments.

PLANT MATERIAL TO BE TREATED

Whole Plants

The treatment of seedlings or small plants can be accomplished with X-rays. Gamma rays can be used to treat small or large plants in a gamma field or gamma room.

Seeds

Seed is the most common plant material treated with radiation or chemical mutagens for seed-propagated species. Seeds are generally preferred because they tolerate physical conditions normally tolerated only by nonliving molecules, such as being desiccated, soaked, heated, frozen, or maintained under varying levels of oxygen and other gases.

Pollen Grains

Pollen grains can be treated with radiation or chemical mutagens. The advantage of treating pollen grains is that the zygote and the plant that develops from it are heterozygous for any genetic change that occurs in the pollen (Fig. 20-1). In contrast, the treatment of seed or whole plants can result in chimeras, in which part of the plant differs genetically from another part. A disadvantage of pollen treatment is that an adequate supply of viable pollen can be difficult to obtain and maintain for some species.

Parts of Plants Used for Asexual Propagation

The treatment of cuttings or apical buds with radiation or chemical mutagens can be effective in developing mutant types in new shoots and plantlets. The important factor is to treat the meristematic region from which new propagules develop.

Cell and Tissue Culture Explants

The use of radiation and chemical mutagens in cell and tissue culture is a rapidly expanding field of research. The concept is to treat single cells or tissue explants, screen them on a medium that will identify the mutant type, and regenerate the desired types into whole plants.

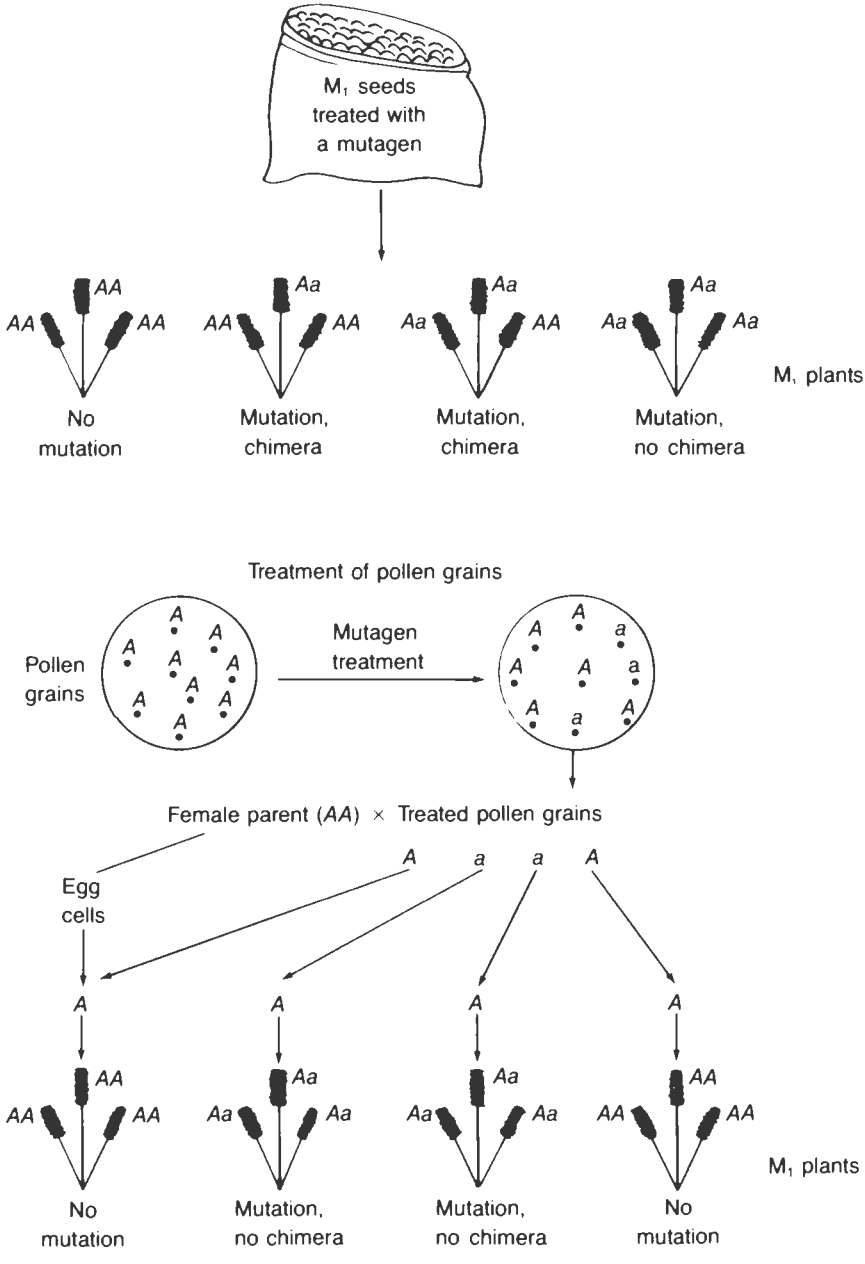

Figure 20-1 Genetic differences between populations of M_1 plants grown from treated seeds and plants obtained from treated pollen grains. The genotype of the untreated seeds is AA and of the untreated pollen grains is A. A mutation is represented by the allele *a*.

FACTORS TO CONSIDER WITH MUTAGEN TREATMENTS

The frequency and type of mutation recovered can vary with the dose and rate at which the mutagen is applied, the species, the genotype within a species, oxygen level, water content, temperature, pretreatment conditioning, treatment conditions, and posttreatment conditions. Each of these variables must be considered in developing a procedure that will be effective.

Dose and Rate

The dose at which a mutagen is applied and the duration of the application are referred to as the dose rate. An acute treatment is one made in a short time, such as a few minutes or hours. A chronic treatment involves exposure to the mutagen for long periods of time, such as weeks, months, or years. The dose rate is a primary variable in a mutagenesis program.

When the desired genetic change involves only a single unit, such as a nucleotide change for a point mutation or loss of part of one chromosome for a deletion, a single exposure to a mutagenic agent for a brief period generally is most effective. When two or more units must be affected simultaneously, such as two chromosomes being broken for a translocation, multiple exposures or chronic exposure generally is preferred.

The appropriate dose rate must be determined by experimentation. For treatment of seeds, the objective generally is to use a dose rate at which 50 percent of the seeds germinate and produce a plant that has viable seed. This is commonly referred to as the LD_{50}.

The most effective way to determine the LD_{50} of seeds is to treat the cultivars of interest with varying dose rates at specified conditions of temperature and moisture, grow the plants in an environment typical of that to be used for later treatments, and count the number of surviving plants. Plant survival for determining the LD_{50} can be estimated from standard germination counts. Such tests can be misleading, however, because plant mortality can occur throughout the growing season. Survival is measured as the ability of a plant to produce at least one viable seed. Germination percentage cannot take into account the number of plants that will be completely sterile. Nevertheless, germination percentage is frequently used to estimate the LD_{50} of chemical mutagens.

Species and Genotypes

There are important differences among species and among genotypes within species for sensitivity to mutagen treatment. Any dose rate study should be conducted on the species that will be used for further mutagen treatment, and preferably on the genotypes to be used.

Oxygen Level

The oxygen level in plant material can influence the amount of damage caused by a mutagen. The higher the oxygen level, the greater the tendency for seedling injury and chromosomal aberrations relative to mutation frequency. The change in mutation effects with oxygen supply is referred to as the oxygen enhancement ratio. To reduce the effect of oxygen, seeds can be treated at high moisture levels or in an oxygen-free atmosphere. In cases where enhanced mutation frequency is desirable, the oxygen effect can be increased by use of dry seeds or by bubbling of air through a solution. For chemical mutagens, the latter is a common practice.

Water Content

The effect of water content is directly related to oxygen supply. High moisture levels reduce oxygen supply, and low moisture levels increase the oxygen supply. Some species are extremely sensitive to changes in water content. Barley seeds at 10.7 percent moisture had a threefold greater response to postirradiation oxygen than seeds at 11.0 percent moisture (Conger et. al., 1968). The effect of water content on mutation frequency is more important for some mutagens than for others. Water content is important in the mutation frequency caused by X-rays and gamma rays. Neither water content nor oxygen supply is crucial when seeds are treated with fast neutrons (*Manual on Mutation Breeding*, 1977).

Temperature

Temperature does not seem to be a crucial factor in radiation treatment but is critical in chemical mutagenesis. The main effect of temperature is on the length of time (half-life) that a chemical is reactive with the plant material. The half-life of a chemical mutagen is the time after which one-half of the initial concentration of the mutagen has reacted. For EMS, the half-life at 40°C is 7.9 hours and at 5°C is 796 hours (*Manual on Mutation Breeding*, 1977). For sulfur mustard, the half-life at 37°C is only about 3 minutes. It is important to know the half-life of a chemical before determining the appropriate treatment procedure.

Pretreatment Conditioning

Adjustment of moisture content to the desired level can be an important part of preparing seeds for radiation and chemical treatments.

Soaking seeds in water is a commonly used pretreatment for chemical mu-

tagenesis. Presoaking leaches out water-soluble substances and hydrates the cell membrane and macromolecules. At temperatures adequate for germination, presoaking can initiate metabolism and DNA synthesis.

When it is preferable to avoid initiation of metabolic activity during presoaking, seeds should be immersed in water at 0°C. It is desirable to keep the water moving by shaking or by having a continuous water flow. The water should be changed every 15 or 30 minutes.

When initiation of metabolic activity during presoaking is desired, the procedure should be the same as just described except that the temperature of the water should be raised and air bubbled through the solution may be desirable. The necessary water temperature and duration of presoaking can be determined by experimentation in which seeds presoaked at different temperatures and durations are exposed to a single dose of a mutagen. The germination percentage and growth of the seedlings will identify the most appropriate treatment.

Treatment Conditions

Temperature of the treatment solution will influence the duration that a chemical mutagen is effective. The pH of the treatment solution can influence the amount of physiological damage and the relative frequency of gene mutations and chromosomal aberrations. There are important differences among chemicals for the pH that is most effective. EMS is commonly used at pH 7.0, whereas sodium azide is most effective at a pH of 3.0. Phosphate buffer of 0.1 M strength or less is recommended if buffers are used in the treatment solution. The use of metal ions in the treatment solution for chemical mutagens is not recommended.

Posttreatment Handling

The handling of treated seeds before planting can markedly influence the amount of survival. Seeds treated with X-rays, gamma rays, or fast neutrons should not be stored more than a few weeks. Oxygen should be eliminated, either by maintaining the seeds at a high moisture level or by placing them in an oxygen-free container. Normal laboratory temperature can be used for storage, unless the seeds are to be kept for extended periods. Seeds should be held at 0°C or less for long-term storage.

With chemical mutagens, posttreatment washing of seeds is recommended to remove residual chemical. Postwashing for 8 hours or more is particularly important if the seeds are to be dried before being planted.

There is a greater rate of survival if moist seeds treated with a chemical

mutagen are planted immediately in moist soil after being washed than if they are dried and stored before planting. No drying procedure has been found that eliminates this phenomenon. If dried seeds must be stored, particularly for extended periods, they should be kept at 0°C or less.

PRECAUTIONS IN THE USE OF MUTAGENS

A mutagenic agent can adversely affect persons who come in contact with it. It is impossible to overemphasize the importance of appropriate safety procedures in using any of the mutagens that are available. The importance of appropriate safety precautions for use of the different types of radiation seems to be well understood. Furthermore, the facilities required for radiation treatments are available in only a limited number of locations and are operated by trained personnel.

Appropriate handling of chemical mutagens is as essential as for radiation treatments. To use chemical mutagens safely, personnel must be aware of the procedures involved in safe storage, handling, and disposal of a chemical mutagen. Many chemical mutagens undergo dangerous reactions upon contact with certain compounds. Gloves, lab coats, safety glasses, pipette fillers, and other equipment should be used to avoid personal contact with mutagens. Procedures and equipment should be readily available to contain and clean up accidental spills in the laboratory or provide medical assistance in the case of human contact with a mutagenic chemical. The *Manual on Mutation Breeding* (1977) describes physiochemical properties, appropriate storage, cleanup and disposal procedures; dangerous reactions; and health hazards of various classes of mutagenic compounds.

BREEDING PROCEDURES FOR SEED-PROPAGATED SPECIES

The plant breeder must make a number of important decisions with regard to development of populations by artificial mutagenesis and selection of mutants within the populations.

Objective of the Mutation Breeding Program

The characters to be changed by mutation breeding should be clearly defined. Selection for one character will have a greater chance of success than selection for two or more characters simultaneously. Efficient methods of screening large numbers of plants should be developed to increase the chance of finding the desired mutant type.

Selection of the Parents

Selection of an appropriate parent depends on the objective of the mutation program. If the objective is to release a cultivar with improved characteristics, the parents should be existing cultivars or experimental lines with favorable characteristics for all traits except the one(s) to be changed. Selection of a parent for such a breeding program is essentially the same as choice of a recurrent parent in a backcrossing program.

When mutation breeding is used to create a characteristic that does not occur in the species, a breeder has two choices in selecting parents. The parent can be an agronomically desirable cultivar or experimental line that is inferior for the characteristic. Alternatively, a parent can be chosen that has the best level of the character, regardless of its other characteristics. For example, mutation breeding is being used in an attempt to lower the linolenic acid content of soybean oil to less than 3 percent. The best adapted cultivars have about 8 percent linolenic acid. Plant introductions with poor agronomic characteristics are available with 5 percent linolenic acid. Use of adapted cultivars provides a chance for release of a cultivar directly if an appropriate mutant type is found, but the amount of genetic change needed to reach the desired objective is greater than for the plant introductions. Use of the plant introductions requires less of a genetic change to meet the goal; however, any desirable mutant types could not be released as a cultivar directly. Whichever type of parent is chosen, several different genotypes should be treated to compensate for possible genetic differences in mutability.

Seed Source of the Parent Cultivars

When mutation breeding is used to improve a characteristic of a cultivar, the seed treated should be representative of the cultivar. A homogeneous supply of a crop, such as from breeder or foundation seed, is desirable to avoid confusion over which off-types are mutants and which are the result of seed mixtures or hybridization. For cross-pollinated populations, the seed source will be heterogeneous and heterozygous; therefore, differentiation between mutants and segregation products may be difficult.

Seed Treatment

Selection of an appropriate dose and rate can have an important influence on the success of artificial mutagenesis. When information is limited as to the type of mutagen to use for a crop species, it is advisable to use two or more different mutagens with several doses of each. A control of untreated seeds should be grown with each generation of inbreeding to provide a comparison for evaluation

of treatment effects. The control seeds should be handled the same as the treated seeds through all procedures, except that they are not exposed to the mutagen. For example, the handling of controls for chemical mutagens should include such procedures as presoaking and postwashing of the seed.

Number of Seeds to Be Treated

The number of seeds to be treated must be based on percentage survival of the M_1 plants, the number of plants that are to be evaluated each generation beginning with the M_2, and the method of handling the treated material each generation. Mutation frequency for a character also is desirable information, but often is not known with precision.

Survival of M_1 plants should be predicted on the basis of preliminary experiments. Laboratory and greenhouse tests may be less severe than field conditions; therefore, survival potential may have to be adjusted according to the environment in which the M_1 plants will be grown.

The number of M_2 plants desired and the method of handling the population during self-pollination will determine the number of M_1 plants required. The number of M_2 plants desired is dependent on the expected mutation frequency. If this cannot be predicted, it is necessary to grow as large an M_2 population as resources will permit.

Growing the M_1 Generation

When a large M_1 generation is desired, the seeds generally must be planted in the field. Survival of the M_1 plants can be influenced by the care taken in planting the seed and in maintaining the planting.

Isolation of M_1 plants from other genotypes of the same species may be important if plants are not to be self-pollinated by hand. For naturally cross-pollinated species, artificial self-pollination is required to obtain M_2 seeds. For self-pollinated species, however, manual self-pollination generally is not conducted and isolation may be important. Mutagen treatments commonly cause some degree of pollen sterility, making self-pollinated species more susceptible to outcrossing. Outcrossing is a problem if it is important to distinguish between character changes caused by mutation and those caused by accidental cross-pollination. Isolation can be accomplished by planting the genotypes an adequate distance apart, by use of different planting dates, by surrounding the genotypes with a different species, or by enclosing the flowers or plants in a bag or cage.

The seeds should be planted at a time conducive to rapid germination and emergence. Unfavorable environmental conditions can inhibit the survival of seedlings weakened by the mutagen treatment. The soil should be friable, moist, properly fertilized to promote plant growth, and at a favorable temperature.

Dry seeds often can be planted mechanically. Wet seeds treated with a chemical mutagen must be planted by hand into moist soil. Rubber gloves should be worn to avoid contact with any residual mutagen on the seed. The soil should be kept moist until the seedlings emerge.

Some species are more easily germinated in the laboratory and transplanted to the field. No selection for vigor of plants should be practiced in choosing M_1 plants for transplanting.

It has been found that mutations are more likely to be present on the main stem than on tillers and branches. The plant density should be high enough to prevent tillering and branching and still permit differentiation of individual plants.

Herbicides can be used for weed control, but they must be nonsystemic ones. A systemic herbicide may influence the vigor and survival of the plants.

In addition to the notes normally taken on breeding plots, additional data on M_1 plants often are desirable. Determination of the percentage emergence of treated seeds, seedling survival, and plant survival to maturity is important in identifying an appropriate mutagen and a proper level of treatment. Notes on the sterility of M_1 plants can provide information on the type of genetic alterations induced by the mutagen and on appropriate procedures for generation advance. Sterility can be estimated from observations made at the time of flowering or through its effect on seed yield.

Breeding Methods from the M_1 to Later Generations

The challenge for the breeder is to handle the M_1 and later selfing generations in a manner that will maintain and identify any desirable mutations that occur. For a mutation to be recovered, (a) it must occur in a cell that gives rise to the reproductive organs from which a seed is produced, (b) the seed containing a mutation must be harvested from the M_1 plants and planted as part of the M_2 and later generations, and (c) any recessive mutation must be homozygous for its phenotype to be expressed.

When a mutation occurs in a pollen grain that fertilizes an egg, all cells of the seed and the plant that develops from it will bear the mutation in the heterozygous condition (Fig. 20-1). Treatment of seed, however, usually causes a genetic change in only part of the meristematic cells. As a result, the plant may have parts that differ genetically, referred to as a chimera. For example, the main stem of a plant may have developed from a mutated cell, while all the tillers or branches developed from an unmutated cell in the seed. If seeds are harvested only from the tillers or branches of the plant, the mutation will not be recovered. The method used to handle the M_1 and later generations, therefore, can influence recovery of a mutation.

The breeding methods that can be used during the selfing generations are the same as those available for a population developed by hybridization. The M_1 and F_1 generations are genetically similar in the sense that plants can be heterozygous at a locus. Populations of M_1 and F_1 plants are quite different, how-

ever, because the M_1 plants in a population can be different genetically, some possessing a specific mutation and others not. All F_1 plants are genetically the same if they were derived from a cross between two inbred parents. A population of M_1 plants must be handled as if it were segregating as an F_2 population.

Each of the breeding methods has advantages and disadvantages for maintaining and selecting mutant types. With a fixed amount of resources for testing M_2 plants, a choice must be made between evaluating multiple progeny from a smaller number of M_1 plants or one or a few progeny from more M_1 plants.

Pedigree Method The steps in the pedigree method for inbreeding a population by artificial mutagenesis are

Season 1: M_1 plants are grown and harvested individually.

Season 2: Sufficient M_2 progeny are grown from each M_1 plant to provide a chance of recovery of a mutant segregate. The method for determining the appropriate number of progeny to obtain for a given probability of success is the same as that described for the backcross method (Chap. 28). M_2 plants that have or seem to have the desired phenotype are harvested individually.

Season 3: M_3 progeny from selected M_2 plants ($M_{2:3}$ lines) are grown. Progeny rows having the desired mutant phenotype and uniformity for other characters may be harvested in bulk. If a row has the desired mutant phenotype but is segregating for other characters, M_3 plants may be harvested individually. The plants may be progeny tested in season 4 and additional selection among and within rows may be conducted in subsequent generations.

Season 4: The performance of uniform $M_{2:4}$ lines may be evaluated in replicated tests for the character under selection and for other important agronomic characteristics.

Season 5 + : In subsequent seasons, desirable lines may be tested for release as cultivars or for use as parents.

The advantage of the pedigree method is that growing progeny from each M_1 plant provides a good opportunity for recovering a mutant if it was present in the M_1 individual. The number of M_1 plants that can be evaluated, however, would be less than in the case of single-seed descent. The labor required for pedigree selection is greater than that needed for the other methods.

Bulk Method The maintenance of a population in bulk requires less labor than any other method:

Season 1: M_1 plants are grown and their M_2 seeds are harvested together as one bulk population.

Season 2: A sample of the M_2 seeds from season 1 are planted. Individual M_2 plants may be harvested for progeny testing in season 3 or all of the

plants can be harvested together in bulk. Mass selection may be practiced on the plants before they are harvested in bulk or on the bulk seed.

Season 3: Two procedures are possible: (a) The M_3 progeny from individual M_2 plants harvested in season 2 may be grown. Subsequent selection among and within progeny may be conducted in the same manner as described for pedigree selection. (b) A bulk of M_3 seeds from season 2 may be planted. Individual M_3 plants may be harvested for progeny testing or alternatively, the plants may be harvested in bulk with or without mass selection of the plants or seeds. The bulk procedure may continue for as many generations as desired.

Season 4+: After a desirable line is recovered, it may be used as a cultivar or parent.

With the bulk method, inexpensive forms of mass selection may be useful in the M_2 and later generations to recover the desired mutation. The primary disadvantage of harvesting M_1 plants in bulk is related to the possibility that the individuals containing a high frequency of mutations, including the desired one, may produce fewer seeds than individuals with no mutations. Thus, when a sample of seed is taken from the bulk to plant the M_2 generation, the chance that the seed comes from an M_1 plant with low productivity is less than the chance that it comes from a highly productive individual.

Single-Gene Descent Productivity of an individual does not influence single-seed descent as long as one or a few seeds are available from each plant. When all of the seed harvested from the M_1 populations is planted in the M_2, each M_1 individual can be represented equally in the M_2, except for differences in seed viability.

Season 1: M_1 plants are grown and one or a few M_2 seeds are harvested from each plant and bulked.

Season 2: The M_2 seeds are planted. Desirable M_2 plants may be harvested individually for progeny testing in season 3 or one or a few seeds may be harvested from each plant and bulked. The seeds may be harvested from only those plants with a desirable phenotype.

Season 3: Two procedures are possible: (a) The M_3 progeny from individual M_2 plants harvested in season 2 may be grown. Subsequent selection among and within progeny may be conducted in the same manner as described for pedigree selection. (b) The bulk of M_3 seeds from the single-seed harvest in season 2 may be planted and individual M_3 plants may be harvested for progeny testing. As an alternative, one or a few seeds may be harvested from each plant and bulked. The single-seed descent procedure may continue for as many generations as desired.

Season 4+: After a desirable line is recovered, it may be used as a cultivar or a parent.

An advantage of single-seed descent is that more M_1 plants can be utilized than with the other methods because only a few progeny are sampled from each individual. This also can be a disadvantage, because by chance none of the few seeds sampled may have the mutation, even though it was present in the heterozygous condition in an M_1 plant.

Early-Generation Testing The primary use of early-generation testing would be for quantitative characters that cannot be selected visually:

Season 1: M_1 plants are grown and harvested individually.
Season 2: $M_{1:2}$ lines are grown in replicated plots. The lines with the desired performance are retained.
Season 3: Selected $M_{1:3}$ lines are grown and individual M_3 plants with desirable characteristics are harvested individually.
Season 4: $M_{3:4}$ lines are evaluated in replicated plots.
Season 5+: A line with the desired characteristics may be released as a cultivar or used as a parent.

When lines derived from plants in the M_1 or later generations are evaluated in replicated tests, the assumption is that segregation for the desired mutation will influence performance sufficiently so that the heterogeneous mutated line will be chosen. Selection within the heterogeneous line at the desired level of inbreeding would identify the desired mutants in the homozygous condition.

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