PART I

ESSENTIAL BASIC INFORMATION

1. AN OVERVIEW OF THE METHODS OF CROP IMPROVEMENT

OBJECTIVES
- To increase yield
- To develop plants that are resistant to pests and diseases
- To improve quality
- To develop plants that have resistance to adverse conditions

Selection – Introduction — Hybridization — Mutation Breeding — Biotechnology

PIONEERS
- 700 BC, Assyrians & Babylonians were already artificially pollinating Date Palm
- 1694, Camerarius - reported sex in plants
- 1717, Thomas Fairchild - Demonstrated artificial hybrid in plants
- 1758-1835, Thomas Andrew Knight - First man to use hybridization for practical plant improvement
- 1866, Gregor Mendel - First auto-ritative establishment of the principles of inheritance
- 1868, Meischer - discovered DNA
- 1900, Hugo De Vries, Carl Correns & Erich Tschermak - rediscover the work of Mendel independently
- 1953, Watson & Crick - discovered DNA materials that carry the hereditary characters of organisms
- 1960-66, Joseph Kolreuter - did the systematic study of artificial hybridization

PIONEERS
- 1896, Becquerel - discovered radiosensitivity
- 1896, De Vries - proposed the use of radioactivity for inducing mutation
- 1927, Muller - showed that x-ray induce genetic deviants in Drosophila
- 1928, Stadler-reported high mutation rates in x-rayed maize and barley
- 1934-35, Herman Nilsson-Ehle & Ake Gustaffson-started mutation experiment in plants & reported erectoids mutants in barley with compact head type & stiffcr straw

PIONEERS

I. 1854 - 1945, Gottlieb Haberlandt - father of plant tissue culture. He was the first person to culture isolated fully differentiated cells and ovary.

II. 1901, Winkler - observed that pollen tubes stimulate growth in ovules and ovary.

III. 1904, Manning - successfully grew excised nearly mature embryo.

IV. 1922, Robbins and Kotte - reported some success with growing isolated root tips.

V. 1925-29, Laiback - demonstrated the practical application of embryo culture in plant breeding by isolation the embryo from a non-viable seeds of the cross *Linum perenne* x *L. austriacum* and reared them to maturity on a nutrient medium.

VI. 1934, White - reported successful growing for the first time continuous culture of tomato root tips.

VII. 1939-50, Street and his Students - conducted an extensive work on root culture to understand the role of vitamins in plant growth and shoot - root relationship

VIII. 1939, Gautheret - first to continuously grew tissue culture from carrot roots.

IX. 1939, Gautheret, White and Nobercourt - were credited for laying the foundation for further work in plant tissue culture.

X. 1941, Van Overbeek and Co-Workers demonstrated for the first time the stimulatory effect of coconut milk on the embryo development and callus formation in Datura

XI. 1963, Raghavan and Torry; 1965, Norstog and others - successfully developed synthetic media for culture of younger embryo.

XII. 1944, Scoog; 1951, Scoog and Tsui - demonstrated that the addition of adenine and high levels of phosphate increased callus and bud formation in tobacco pith tissue culture.

XIII. 1953, Muir - demonstrated that transferring callus of *Tageta erecta* and *Nicotiana tabacum* to liquid medium and agitating the culture on a shaking machine broke the tissue into single cells and small cell aggregate.

XIV. 1954, Muir et al, - succeeded in mechanically picking single cell from the shaked culture as well as callus tissues and making them individually on separate filter resting on the top of the well-established callus-culture.

XV. 1960, Jones - designed a micro-culture method for growing single cells in hanging drops in a conditioned medium.

XVI. 1960, Cocking - demonstrated the feasibility of enzymatic degradation of cell walls to obtain large quantities of viable naked cells called protoplast.

XVII. 1965, Vasil and I lildebrandt - raised whole plants starting from single cells in tobacco.

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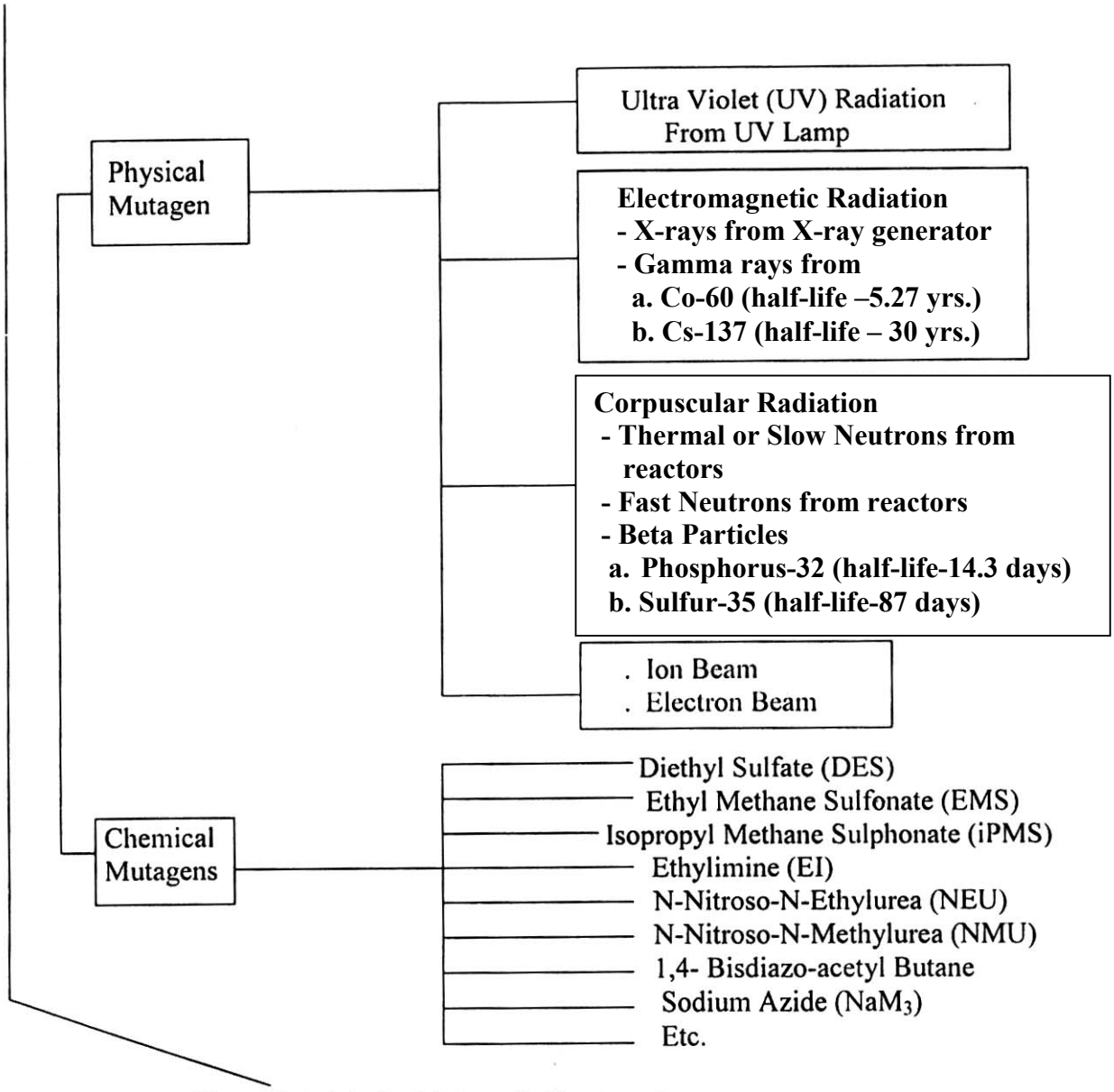


. Seeds
. Pollen
. Whole Plant
. Cuttings
. Tubers
. Corms
. Bulbs
. Stolons
. Cultured Cells and Tissues

2. MOLECULAR BASIS OF MUTATION: THE NUCLEUS, CHROMOSOMES AND DNA

2.1 THE NUCLEUS

Cells whether of animal or plant (except for very few exceptions) are composed of two most important principal recognizable parts, i.e. nucleus and cytoplasm. The nucleus is bounded by a membrane within the cytoplasm. It is separated form the cytoplasm by a membrane. Generally, these two major parts of a cell are function-wise separate. The nucleus coordinates and directs cellular activities whereas the cytoplasm carries out the directions made by the nucleus. The cytoplasm is the site of metabolism in the cell, i.e. building up of protein and other essentials and the tearing of worn out structures with the consequent production of waste materials. But the nucleus orders times and directs this metabolism. The separation of the nucleus and cytoplasm does not imply independent, but are dependent upon each other. Neither could survive without the other. Events happening in either the nucleus or cytoplasm will have important effects in the other major parts of the cell. Changes occurring in the nucleus can alter its ability to direct metabolism in the cytoplasm. Likewise, alterations in the cytoplasm or even in its membrane that surrounds it, can exert an effect within the nucleus since it is the metabolic activity of the cytoplasm that supplies the materials the nucleus needs for its own existence, i.e. its repair and reproduction and its function of coordination and direction of cellular activity. Hence, due to its intimate interdependent changes detected in the nucleus in structure and function may have been due to events taking place in either itself or in the cytoplasm and vice-versa. Changes in the cytoplasm that bring about anomalies in nuclear material will almost unavoidably have further consequences in the cytoplasm. An injured nucleus, no matter the cause of the anomaly or change will not be capable of properly directing cytoplasmic activity. Changes in cytoplasmic function then may be the result of impairment of the nuclear function which may have resulted due to an injury in the cytoplasm.

2.2 THE CHROMOSOMES

The apparatus within the nucleus that directs nuclear activity are the chromosomes. They are present in most cells in specific and fixed numbers. The number of chromosomes in a cell is characteristic of the species. All members of a species carry the same number of chromosomes in every cell. The germ cells (male and female gametes) and the somatic cells (the cells composing the body) differ in chromosome number. The gametes have half the number of chromosomes (n) found in the somatic cells. The somatic cells have twice (2n) as the gametes. The chromosomes which constitute the n number is said to be a set of chromosomes. The n number is made up of a particular number of chromosomes each of which is different from the other. The chromosomes will often differ among themselves in size or shape. More importantly, they may differ in content. All of them taken together form a set, a complete of chromosomes, the n set. It is very necessary for in most organisms, it is a minimum requirement for life. Cells that in some way lose chromosomes or even only parts of it, so that the basic set number is incomplete, cannot live. Even if the cell has more than the n of chromosomes, it will not live if the set is incomplete. The somatic cells have twice the number of chromosomes as are found in germ cells. They actually have two n sets of chromosomes, i.e. each chromosome in the n set of the germ cells in present in somatic cells in duplicate. The symbol used is 2n and the somatic cells are said to have the 2n number of chromosomes with the knowledge that they really represent n + n sets. In man, 2n = 46, n = 23; in onions 2n = 16, n = 8; Tradescantia 2n = 12, n = 6, etc). The chromosomes are the structures which carries the genes. They are the vehicles of the molecules that direct the metabolism of each cell and hence of the total organism. In order to have a complete set of genes, and to have the proper balance between genes for metabolism to be carried out properly, a 2n set of chromosomes is a fundamental requirement. Loss of a chromosome or loss of even a small part of a chromosome will almost inevitably result in the loss of one or more genes, which may produce metabolic anomalies, for unless another is present to take over the function of the lost one, the function itself will, in most likelihood disappear. Or worse, if more are lost,life is impossible. The chromosomes are generally characterized morphologically by the position of the centromeres (the primary constriction) and according to its size (Fig.I-1a, b)

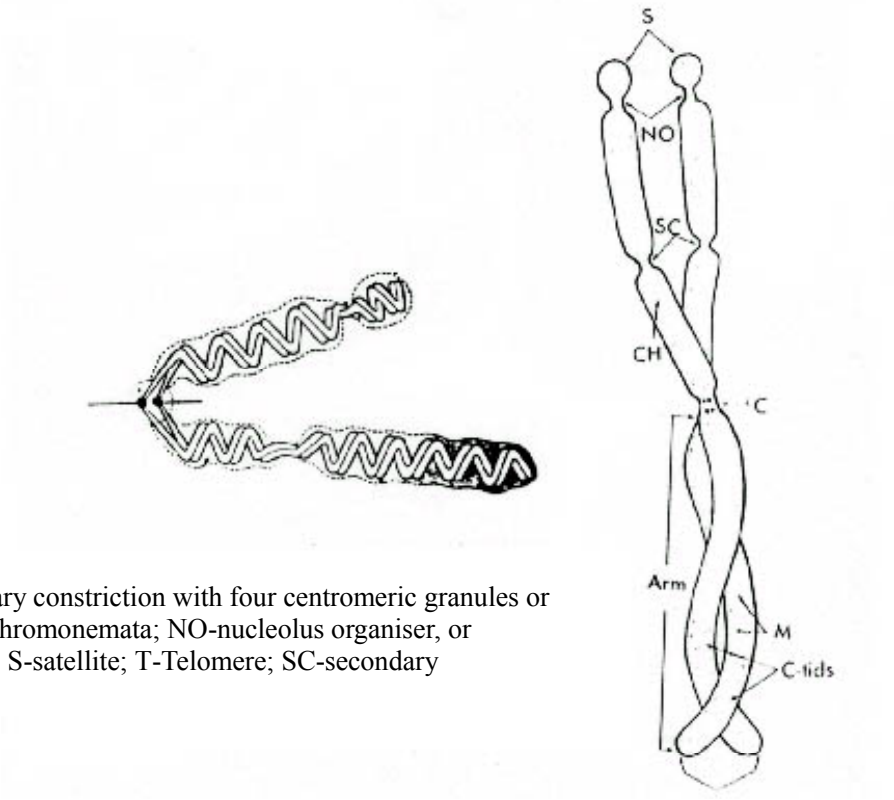


Fig. I-1a
C-Centromere or primary constriction with four centromeric granules or spindle sphericle; Ch-chromonemata; NO-nucleolus organiser, or secondary constriction; S-satellite; T-Telomere; SC-secondary constriction; M-matrix

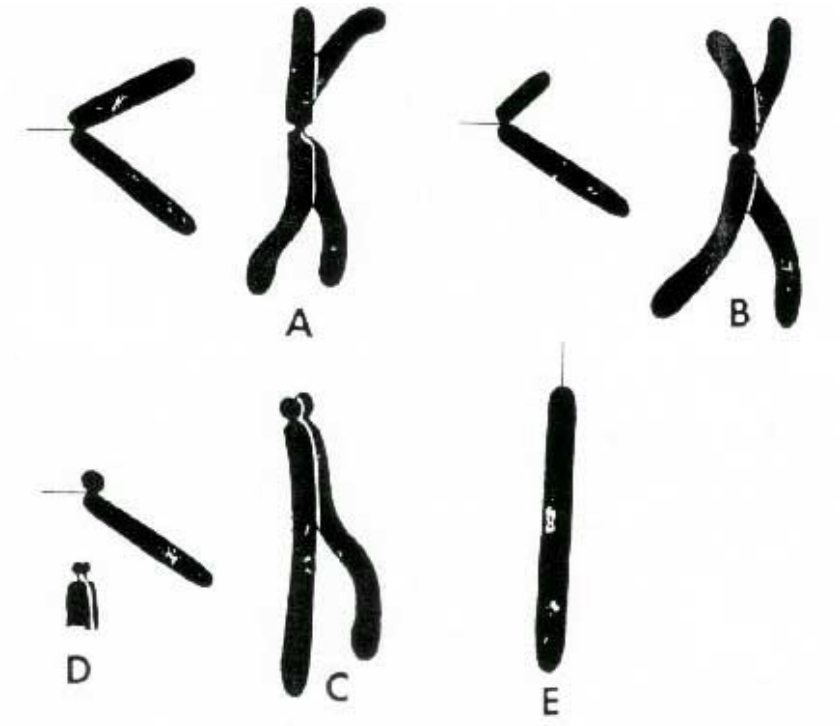


Fig. I-1b Mitotic metaphase chromosome
A- Median position-Metacentric; B- Both arms nearly but not quite equal-Submetacentric; C- If short arm is approximately spherical, has the diameter of the long arm subaciocentric; D- short arm can be seen, has a diameter clearly less than the diameter of the long arm-acro- centric; E- Telocentric, no observable short arm whatsoever.

2.3 THE GENES

The structures in the nucleus of the cell of which the chromosomes are the vehicle which control inheritance and metabolism are called the genes. The genes are composed of very large molecules called

deoxyribonucleic acid (DNA). The type of molecules of which DNA is a representative is called a macromolecule. The macromolecules are made up of subunits which are stable molecules with characteristics peculiar to themselves. It is a large molecule, having certain characteristics, which is made up of smaller yet discrete molecular units. DNA as in most other macromolecules, the subunits of which the whole is composed of, are joined together end-to-end to form a long chain, in what is called polymerization. Such molecules are said to have been polymerized. DNA is usually described as a long-chain polymer, a olymer composed of many subunits connected end-to-end as links in a chain. Each molecule of DNA is composed of two unbranched threads. The two chains or threads are joined in adder-fashiion. The DNA is twisted or spiral structure reseming a spiral staircase. The lnkage fo the subunits in the chains and the chains to each other is very important, as its is the basis of the mechanism by which genetic information is coded and stored in the cells, as well as the basis where this coded information is transmitted to the cytoplasm. It is also important in the proper carrying of genetic information from one cell generation to the next.

2.4 THE DNA

The subunits of which DNA is made up of are themselves composed of phosphoric acid, the five carbon sugar, deoxyribose, and an organic, nitrogenous base. These components taken together are called nucleotides, specifically deoxyribonucleotides. It is these nucleotides that are arranged in sequence in each chain making up DNA. In the macromolecule (the double chain) the side pieces or backbones of the ladder are sugar-phosphates, while the rungs are nitrogenous bases. Only four nitrogenous bases are commonly associated with DNA. They are categorized as purines and pyrimidines. Close examination of the DNA will show that each is made up of nucleotides which differ only in their nitrogenous bases. This difference is specific and constant. Whenever adenine occurs in one chain, thymine will be in the opposite chain. Whenever cytosine occurs in one chain, guanine will occur in the opposite chain. Hence DNA chains have polarity or direction. In each DNA macromolecule chain, a complement appears in the opposite chain. In most molecules of DNA in nature, there will be equal amounts of adenine and thymine and equal amounts of guanine and cytosine, as they occur in pairs. The two chains of the DNA macromolecule are held together by a very weak form of chemical bond called a hydrogen bond, where two molecules share one hydrogen atom and are held together by the atom they share. In DNA, each base pair shares two or three hydrogen atoms so that each is bonded at two or three points, where the chains are joined, the rungs of the ladder are complete (Fig. I-2a, b).

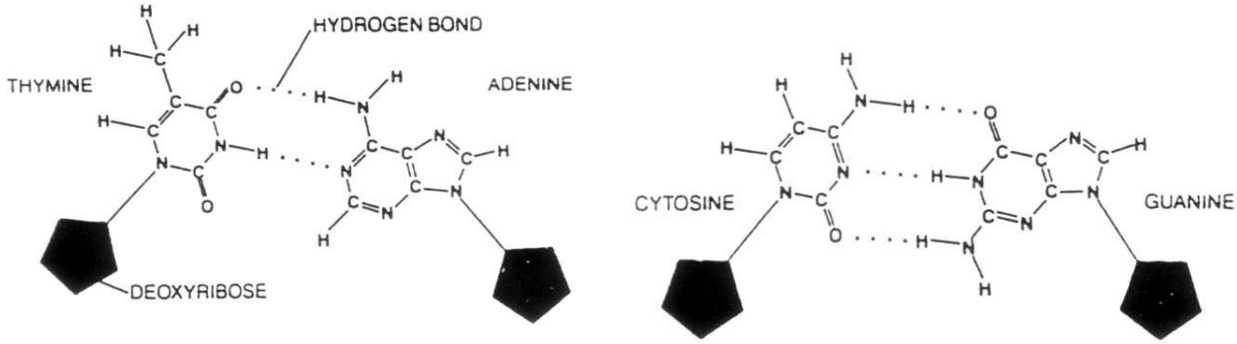


Fig. I-2a Hydrogen-bonding between the bases as it occurs in DNA

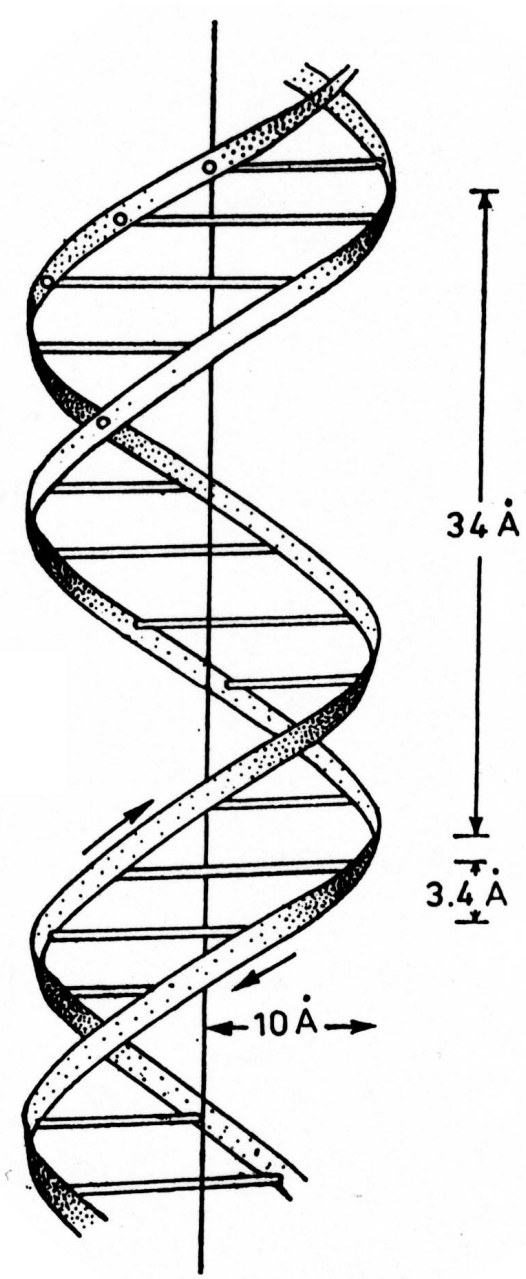


Fig. I-2b The double helix model of DNA

Cellular metabolism as stated earlier is under the control of the gene. The genes and consequently, the DNA which makes them up, direct all the activities of any given cell, hence the DNA must contain all information necessary for the activities of any given cell to direct those activities. A cell's activities include, not only the chemical reactions going on within it but will also include whatever intercellular interactions in which the cell might participate. Such information is indeed present and is stored in the DNA molecule. The sequence of the nitrogenous bases, the purines and pyrimidines along both sides of the DNA macromolecule is the genetic information. The sequences of the purines and pyrimidines along the strands of the DNA polymer is, in a highly distilled or modified mannier, i.e. containing all of the information required by the cell for all its metabolic activity. The sequence or linear arrangement of these bases contains the genetic information and not the bases themselves. It is the linear arrangement or sequence that holds the genetic information. Hence any force which changes this sequence will produce a change in the information stored in the molecule. Such changes will almost always result in an important change in the metabolism of the cell in which they happen. The changes are called mutations and the individual that bear them are mutants.

Mutations have been known for many years as a naturally occurring phenomenon. Almost every species of plant studied, including the very simples, is known to have members arise in its midst which differ from the rest. These are mutants which occurred after a change in the genetic material. The changes and the mutant individuals appeare apparently at random in any population of living things. No cause is now known that explains their appearance. As a result, they have been called spontaneous mutations.

Spontaneous mutations are permanent, heritable changes in individual genes. When genes mutate, the change will, for any given change in any given gene, a rate event, sudden and in discrete fashion, i.e., the gene will pass from one state to another, both of which are stable indicating the genes undergo a definite transformation when they mutate, and that, between mutations they are fixed in composition. The appearance of spontaneous mutations then is an indication that the genes have a distinctive chemical structure, which, like any chemical change, is subject to the all-or-nothing rule.

All somatic cells within an individual contain the 2n number of chromosomes. The genes (or the DNA) are actually part of the chromosomes, and a typical somatic cell contains the 2n number of genes and the 2n amount of DNA. When cells have reached the point where they need to divide it will be necessary for the daughter cells of this division to be provided with the 2n number of chromosomes and with the 2n number of DNA. If this provision were not made, the daughter cells resulting from the division of a mature somatic cell might have different numbers of chromosomes and genes and a different balance between genes. Daughter cells would differ from their parent cell and other cells in the body of which they are part of. To preserve the 2n quantities and balance relationship between genes, the number of chromosomes and the quantity of DNA in the nucleus, must double just before cell division. The DNA and the chromosomes duplicate themselves. Before the cell division, the cell about to divide will have the 4n number of chromosome and the 4n quantity of DNA. Half of this 4n quantity (two copies of each set) will go to each daughter at division, paving the way for each daughter cell to receive the proper 2n amount of genetic material and have genetic balance similar to its parent.

When the DNA duplicates itself, the hydrogen bonds which link the chains of the DNA molecule break, and the chains separate. Each then directs the synthesis of the complementary chain, the chain similar to the one from which it just separated. The synthesis of the new DNA (the complementary chain) begins at one end of an existing chain and continues to its terminus closing zipper-like. In the nucleus, all of the four nucleotides will be available for the existing DNA to incorporate into the newly synthesizing complementary chain. As a new chain is synthesized, only the proper nucleotide, the right member of the nucleotide pair will occupy each place in the new chain. Only the particular bases, i.e. the proper member of each pair can form a hydrogen bond with the other member of the base pair while at the same time presenting the phosphate group of the new nucleotide in the proper position to be bonded in the growing new chain. DNA synthesis under ordinary circumstances is really DNA duplication, for the new DNA is identical to the old, i.e. no change comes about. Hence, at cell division, each daughter cell which provided no error in the DNA transmission will get exactly the same genetic information as its parent had.

Another sequence of events, however, may take place in rare situations where the electrons and the protons of the molecules of nitrogenous bases can undergo rearrangement. The distribution changes within these molecules so that they actually have more than one possible structural configuration. The various forms have the same number of protons and electrons, they are however, arranged somewhat differently in each molecule, resulting into the rearrangement of the parts of the molecule called a tautomeric shift. The rearranged structural configurations, where molecules may exist are called tautomers. One tautomeric form will be, by far, the most commonly occurring one. Most of the molecules of these bases will exist in this form. But sometimes on rare occasions, an internal arrangement (a tautomeric shift) will take place in another molecule in a common form to produce another uncommon form, a rare one. These shifts are not necessarily permanent. These rare forms can undergo rearrangement either to other rare forms or back to the common one. When a tautomeric shift on one of the bases in a nucleotide occurs during DNA synthesis, the resultant new DNA may not be a duplicate of the old. It may differ from the old in some very significant detail. Many consider this phenomenon of tautomerism of the nitrogenous bases as the molecular basis of the mechanism of mutation.

The base when it is in its common form during DNA synthesis can form a hydrogen bond with only one of the other bases, that is adenine-thymine, guanine-cytosine and yet presents each phosphate group in the proper position for bonding in the molecule. But if one of the bases should be in a rare tautomeric state during DNA synthesis, it may not be able to form a hydrogen bond with its partner, i.e. adenine will not be able to pair with thymine and neither will guanine in its rare form pair with cytosine in their tautomeric states. They will find paring in the DNA with adenine and guanine difficult. The rare tautomers will be able to pair though with the base of which pairing is forbidden, i.e. rare adenine pairs with cytosine and rare thymine with guanine resulting in directing the incorporation of the wrong partner, the forbidden partner, into a new DNA chain at the level of rare tautomer. The sequence of bases of such rare tautomers will be different from the sequence of the old chain so that the genetic information may be drastically changed. Although at this stage of events a full blown mutation does not as yet exist, it is a transition stage between the old and the new, and the inception of a possible mutation. The rare tautomer in the old chain will probably return to its common state and is not permanent. Change will have ensued in that part of the molecule. In the new DNA chain, the sequence of the bases is permanent. The genetic information it represents is inheritable. Such a sequence of events take place in a cell giving rise to gametes, the mutation will be handed on to the future generations of the individuals through the gametes. When the cell that inherits this changed DNA chain prepares to divide the chain will, of course, direct the synthesis of a chain complementary to itself. It will do so according to the permitted (altered) pairing of bases, so that the complementary chain synthesized will be different from the one that made them before. Thus, this change too is inheritable. Both chains of DNA have undergone the change and the mutation is said to be accomplished and completed.

3. MUTAGENESIS

In nature, sudden, rare, discrete changes in the genetic material which result in a permanent change in the expression of the genes take place spontaneously may be due to an interplay of the combined effect of background radioactivity, extremely high or low temperatures, presence of chemicals and age of seeds and plant nutrition. These sudden heritable changes in plants or animals are called mutation and may be classified as spontaneous or induced, somatic or genetic, chromosomal or extra-chromosomal. Although mutations occur spontaneously with fixed frequencies, it does not mean that the frequency of each occurrence is not changing. Certain agents, known by its generic name mutagens can change the rate of its appearance. Mutagens as the name implies, (mutation-change; genesis – to give rise to or generate change) as an agent of mutation can increase its frequency and rate. There are a number of fundamentally different types of mutagens completely unrelated in structure and mechanism of action. A large general type of rich diversified agents can be found in chemical mutagens, can change the genetic material of incorporated cells. Ionizing and those of lesser energy (ultraviolet) radiations are mutagens that are quite efficient for producing changes in genetic material. But it must be emphasized that mutation is basically a property of the genetic material and not a property of the mutagens themselves. Since mutagens are so different from each other both in what they are and how they act, mutation is probably a response of the genetic material common to any agent that produces ultramicroscopic, point-like discrete changes in it.

3-1 RADIATION MUTAGENESIS

It has been suggested that ionizing radiation acts by ionizing nitrogenous bases in the DNA chains in particular during the DNA synthesis in the production of mutations. If ionization of the base occurs during DNA synthesis, forbidden base pairs can come into existence in the new macromolecule just as they are formed following tautomeric shifts in the structure of the bases. As a result, the sequence of bases in the newly synthesized chain would be wrong, and a permanent, heritable, discrete change in the DNA would be produced. This change(s) or mutation would be similar to the one that occurs spontaneously, but the frequency of its appearance would have been increased. Aside from ionization of the bases of DNA, base change or base deletions, effects that can change critical base sequences of the molecule, may occur. If sufficient numbers of ionization take place within one or more bases or if interaction with free radicals so alter one or more bases they are deleted from the DNA molecule. Base deletion occurs.

There are available a number of radiation types and radiation sources to mutation breeders. Aside from ultraviolet, several types of ionizing radiation, i.e. X- and gamma rays, alpha and beta particles, protons and neutrons, which has in common the ability to form discrete releases of energy called ionization or ion pairs, as they pass through matter. X-rays, like gamma rays and the UV light are electromagnetic radiations emitted as quanta ($10^{0.001nm}$ for X- and gamma rays in contrast to 2,000 to 3,000 nm for UV light). In an X-ray machine electrons are electrically accelerated in a high vacuum and then stopped abruptly by striking a target of tungsten or molybdenum, the deceleration of the moving electric charge is accompanied by the emission of radiation. For mutation induction it is desirable to use X-ray machines producing short wavelength radiations (hard X-rays) as the penetration is greater than with longer wavelengths. The short wavelengths emitted is related to the peak operating voltage (kVp) of the tube. The higher the kVp, the shorter the wavelength of the X-ray that is produced. When X-ray irradiation is used, voltage (kVp), current (mA), thickness and type of filter, distance of the tube to target, dose and dose rate should be noted as well as the half value layer (expressed in millimeters of aluminum or copper) i.e. the thickness of an absorber required to attenuate a beam of radiation to one half. This value is the measure of the effective energy of the X-ray machine or the quality of the beam.

3.1.1 GAMMA RAYS

Gamma rays have generally a shorter wavelength and hence possess more energy per photon than X-rays. Gamma radiation in usually obtained from radioisotopes in contrast to X-rays. A gamma radiation facility can be used essentially in the same manner as an X-ray machine for acute or semi-acute exposures. The most distinct advantage of gamma radiation source for prolonged treatments is that it can be placed in a greenhouse or field so that plants can be exposed as they develop over long periods of time. Cobalt-60

and Cesium -137 are the main sources of gamma rays used in mutation breeding. They are stored in lead containers when not in use and operated by remote control mechanisms to irradiate plant material.

3.1.2 UV RAYS

Ultraviolet light has limited penetrating ability, therefore its use is limited to treating spores, pollen grains cells and cultured tissue. Wavelengths in the range of 2,500 to 2,800 nm are biologically most effective because this is the region of maximal light absorption by nucleic acids.

3.1.3 BETA PARTICLES

Beta particles such as those from Phosphorus-32 and Sulfur- 35 produce effects in tissues similar to those of X- or gamma rays. The penetrating ability of beta particles is lower than that of X- and gamma rays. The lower penetrating ability of beta particles can be overcome by putting the radioisotope in a solution and administering them to the plant material. P-32 and S-35 may then be incorporated directly into cell nucleus giving a somewhat greater localization of the site of action. But, because of the variability from tissue to tissue and cell to cell, it is difficult to determine the exact dose given by an internal emitter in plant material.

3.1.4 NEUTRONS

Neutrons have been shown to be highly effective for the induction of mutation in plants but, a certain degree of confusion exists concerning the results of early experiments and due to lack of adequate dosimetric techniques.

3.1.5 ION BEAMS

Ion beams can give a large amount of energy with high LET (Linear Energy Transfer) to the localized position in tissues. Therefore, we can expect different biological effects will be given to plant materials compared with low LET radiations, such as, gamma- and x-rays. Also ion beams can produce large structural changes in chromosomes and DNA. So we can expect to induce different kinds of mutations in plants with high frequency than gamma- and x- rays. But further basic and practical researches should be made to use ion beams efficiently in the future.

3-2 CHEMICAL MUTAGENS

The number of chemical mutagens is numerous and continuously increasing. But for mutation inductin in cultivated plants only a few are readily very useful and most of them belong to the special calss of alkylating agents such as, ethyl methane sulhonate (EMS), diethyl sulfate (dES), ethyleimine (EI), ethyl nitroso urethane (ENU), ethyl nitroso urea (ENH), and methyl nitroso urea (MNH). A number of workers also found azides as effective mutagens.

3.2.1 BASE ANALOGUES AND RELATED COMPOUNDS

True base analogues are closely related to DNA bases, adenine, guanine, cytosine or thymidine and can be incorporated into DNA without affecting its replication. But analogue differs from the normal base in certain substitutes hence its electronic structure is modified and it can be expected that occasional pairing errors will occur at the time of DNA replication after the analogue has been incorporated. The most frequently used analogues are 5-bromo-uracil (BU) and 5-bromo-deoxyuridine (BUDR), which are analogues of thymine. Apart from true analogues, it has been found that N-methylated oxypurines have a chromosome effect. The most efficient compounds are 8-ethoxy caffeine (EOC) and 1,3,7,9 tetramethyl-uric acid (TMU), but their mode of action is still unknown.

Maleic hydrazide (MH), an isomer of uracil, induces chromosome breaks in cell and aberrations are localized in heterochromatic regions near the centromere of the chromosomes.

3.2.2 ANTIBIOTICS

Antibiotics such as azaserine, mitomycin C, streptonigrin and actinomycin D have been found to have chromosome breaking properties, but their usefulness are limited.

3.2.3 ALKYLATING AGENTS

This is the most important group of mutagenic chemicals for mutation induction in cultivated plants. They have one or more reactive alkyl groups which can be transferred to other molecules. They react

with DNA by alkylating the phosphate groups as well as the purine and pyrimidine bases. One should be extremely careful in using them because most are potential carcinogens, such as EI, EMS, MNH and should be used in small units.

3.2.4 AZIDE

Azide is an effective mutagen under certain treatment conditions. It is possible to obtain high mutation frequencies with azide. Most mutations are gene mutations with some minor frequencies of chromosome aberrations. It is relatively safe, non-persistent and inexpensive but are also potential carcinogens.

4. OTHER MECHANISMS OF MUTAGENESIS

Mutations are found to occur without any apparent cause and are usually referred to as spontaneous mutations. A closer analysis of the origin of spontaneous mutations showed that factors both intrinsic and extrinsic to the organism may be responsible both for spontaneous mutations and for increased frequencies over the spontaneous mutation rate. The important intrinsic factors are genetic constitution and physiological conditions and the important extrinsic factors are nutrition, temperature, natural radiations and chemicals and very high oxygen pressure.

4.1 GENETIC CONSTITUTION

Spontaneous chromosome structural changes with high intensity and frequency has been alluded to either hybridity, polyploidy or either both during meiosis or mitosis.

4.2 PHYSIOLOGICAL CONDITIONS

It has been found that sex and age influence on spontaneous mutation rate.

NUTRITION

Nutrition is known to influence spontaneous mutation rate in many plants. Spontaneous mutability is increased by sulphur, phosphorus and nitrogen deficiencies. In plants grown in a magnesium or calcium deficiency, a very high incidence of chromosome breakage occurs in the order of up to seventeen times the control values in the case of calcium deficiency. An extensive analysis on the effects of deficiencies of essential elements has shown that radiosensitivity is increased by zinc deficiency and to a lesser extent by copper, sulphur and molybdenum deficiencies.

TEMPERATURE

In higher plants, dry seeds subjected to heat treatment induced chromosomal aberrtions and mutations. The mutagenic effect in the heat treatments is so low to be useful in mutation experiments.

NATURALLY OCCURING RADIATIONS

It was thought that natural radiation may be responsible for spontaneous mutations in plants. However, it can not be excluded that mutations may occur at increased rate in organisms living in areas with a markedly increased natural radiation such as exists in India, Brazil, Southern France and other countries unless hormesis exists.

NATURALLY OCCURRING MUTAGENS

Naturally occurring metabolites of unknown composition, sulphur-containing substances, amines, amino acids, amides, nitrogen-free acids, aldehydes, alkaloids, phenols, quinines, degradation products of nucleic acids and the widespread applications of chemicals in present-day civilization and the industrial pollutions from factories, motor vehicles, energy production, etc. the genetic implications of chemical mutagens may tend to increase.

5. CELL CYCLE

THE NON-DIVIDING NUCLEUS

The non-dividing cells, whether interphase or postmitotic stage is not characterized as much by observable structures as by physiological activities especially the nuclei part. At the end of mitotic telophase chromosomes practically disappear into the non-dividing, the energic nucleus. The term energic emphasizes its biochemical activity and at the same time does not imply that it is neither interphase or postmitotic. Non-dividing nuclei and cells are of two sorts, i.e. those in the interphase, which are preparing for another division and those at postmitotic which will normally not divide again. The postmitotic do not replicate their DNA during the synthetic phase but differentiate in a permanent Gap 1 (G_1) condition.

In eukaryotic cells one or more nucleoli forms in each telophase nucleus. Nucleoli are formed by specific regions of specific chromosomes called nucleolus organizers while which are usually located in short arms of certain chromosomes. If they lie near the ends of chromosome arms, the tip of the arm beyond the nucleolus organizer constriction is called a satellite. Nucleolus organizers are usually detectable only at metaphase in higher plants. In a cell of a diploid plant species there are at least two, one in each chromosome set. Nucleoli are large nuclear organelles that are not bounded by memberane, usually lie in the nucleus and are rather spherical. The nucleoli represents the fist phases of ribosomal formation. The DNA genes (cistrons) of the nucleus organizer region (rDNA) aided by RNA polymerase transcribe RNA (rRNA). Protein appears to be synthesized immediately in the nucleolus. The chromatin within the non-dividing cell nucleus is mostly invisible by the optical microscope. The biological significance of the nondividing cell nucleolus is its physiological activity which has been worked out in considerable detail during the past 50 years or so.

THE CELL CYCLE

It is common knowledge that during the interphase between two mitotic divisions the DNA and histones replicate during the synthetic (S) period. The time between the preceding telophase and the beginning of the S period is called the Gap 1 (G_1) period and the time between the end of the S phase period and the beginning of the prophase is the Gap 2 (G_2) period. The mitosis itself is called the M-period. In diploid organisms the relative amount of DNA in a diploid telophase and during the G_1 period is 2C and from G_2 to telophase a diploid cell has the 4C amount of DNA (Fig. I-3).

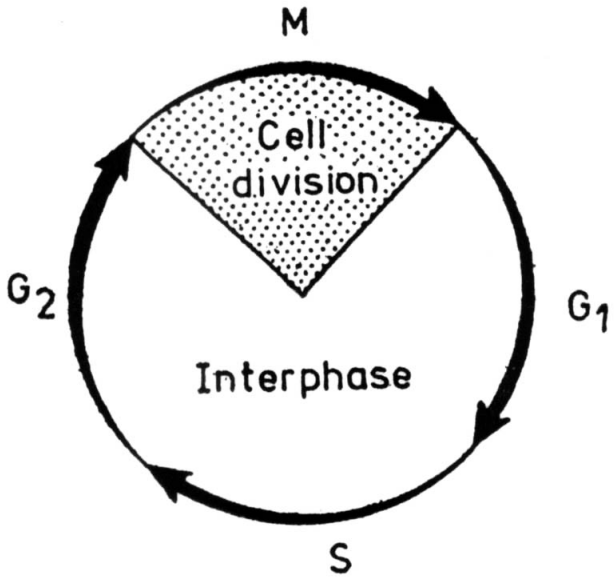


Fig. I-3 The cell cycle

5.1 MITOSIS

Next to DNA replication, mitosis is the fundamental form of reproduction in all organisms. Life can exist at no level lower than the complete cell with its hereditary and informational DNA, its organelles, enzymes, and other material needed for all the numerous processes necessary for life. Mitosis generally divides this mass into more or less equal and self-sufficient daughter cells after the DNA and other essentials have been exactly or approximately doubled. During the interphase following mitosis, the DNA is exactly doubled quantitatively and qualitatively by the semi-conservative process, followed right away or even later by structural doubling into chromosomes consisting of two cytogenetically identical chromatids. Followed by events that divide the chromatids into two identical groups and the cytoplasm is divided also into two very similar portions. Hence, the daughter cells almost always are genetically identical to each other. And each is identical genetically to the cell where it came from. Cells may enter mitosis from interphase rather inevitably or cells in the post mitotic condition may be stimulated naturally or by treatment to do so. In the case of post mitotic cells some sort of inhibition must be removed to stimulate DNA replication by the application of auxin. (Fig. I-4)

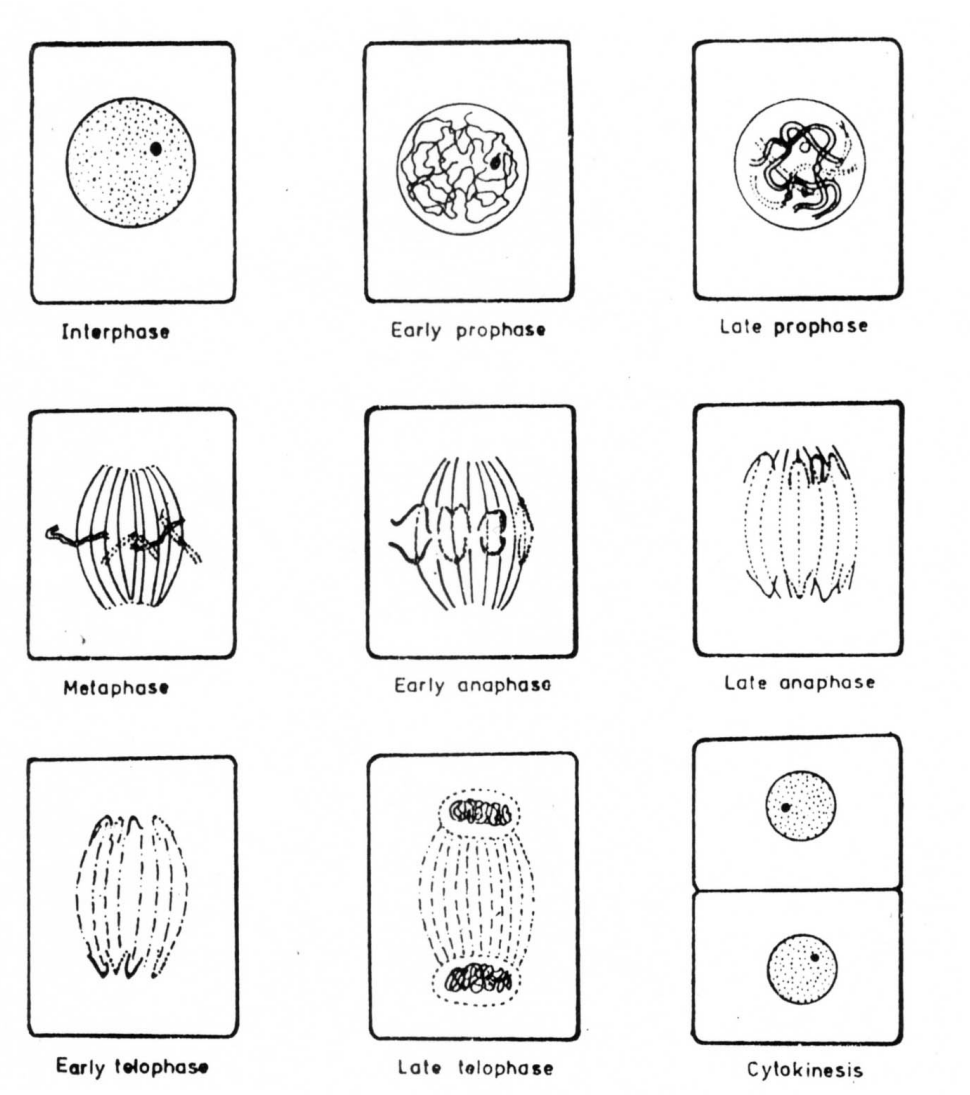


Fig. I-4 Diagrammatic representation of different stages of mitosis

PROPHASE

The onset of nuclear division is characterized cytologically as the gradual disappearance of the chomatinic network coincidental with the emergence of the long thready chromosomes. Throughout

prophase, the chromosomes become progressively shorter and thicker as a result of coiling as if each chromosome is a coiled spring enclosed within matrix. At mid-prophase, each prophase, each chromosome is seen to have two chromatids. Each chromosome has poorly staining portion (the centromere) which will be the site for the production of the spindle apparatus at metaphase.

PROMETAPHASE

By the end of prophase in typical plant cell mitosis, the nucleolus and the nuclear membrane disappears. This relatively very short stage of mitosis has been termed prometaphase where the axis of division is evident and the spindle is forming and the chromosomes are nearly as short as they ever will be. Prometaphase is the transition period from prophase to metaphase called metakinesis. It is a dynamic period when the centromeres of the chromosomes (or chromatids) acquire spindle fibers to opposite poles and during which the chromosomes are moved to their metaphase position, i.e. equidistant from the two poles of the spindle. The movement is the orientation of the chromatids in the region of the centromere such that one of them lies toward each of the spindle poles.

METAPHASE

The alignment of the chromosomes in a plane formed by their centromeres midway between two polar bodies of the spindle apparatus characterizes metaphase. The spindle apparatus is basically a complex of fibers extending from pole to pole and from poles to centromeres. At metaphase, the chromosomes of the set are not always arranged at random, the centromoeres lie toward the center of the plate with its arms directed outward. Those at one pole will be included in one nucleus hence at metaphase the spindle continue to develop.

ANAPHASE

The separation of the sister chomatids of each chromosome at the centromere signals the beginning of anaphase. The centromeres of the anaphase chromosomes lead toward the poles. The arms seem to follow passively or to be dragged. Often the ends of the arms of the sister chromatids seem to stick together so that they must be eventually be pulled apart. There is one anaphase error that is of cytogenetic and biological importance, the nondisjunction. This happens when both sister chromatids of a metaphase chromosome end up in same telophase nucleus. It may be a regular gene-determined process or it may be an error which may later produce cells that are aneuploid if one or a few of the chromosomes of the set undergo nondisjunction. Anaphase is usually very regular but when it is not, there is any chromosomal breaks and reunions so that an anaphase chromatid is formed with two centromeres and another without centromeres. And if the two centromeres go to the opposite poles, a bridge is formed along with an acentric fragment. Pieces of unicentric chromosome without centromeres are produced, because of the absence of centromeric spherules. These and their contained genes are not included in other daughter cells and may give rise to micronucleus outside the main nucleus.

TELOPHASE

It is characterized by the conversion of anaphase into interphase chromosomes, the reformation of nuclear envelope, formation of a second centriole, formation of one new nucleolus or more, disappearance of spindle and astral fibers and the division of the cell into two complete and distinct cells, the two daughter cells. The nucleoli become enlarged. Within each nucleus, the chromosomes which started elongation at anaphase become progressively longer and thready until the telophase nuclei merge into resting nuclei.

5.2 MEIOSIS I

Meisos evolved in part as a chromosomal number reduction process and in part to produce recombination. It evolved out of mitotic divisions one or more times but has remained very sensitive to environmental conditions as well as to mutations that act various times and to other anomalies such as hybridity may create. Many variations of it have evolved as normal for certain species, such as various forms of apomeiosis, non-disjuntion, directed chromosomal eliminations, multiple or unipolar spindles etc. Sexual reproduction in eukaryotes involves the production of nuclei, with the haploid chromosome number and the means for delivering the diploid chromosome number in the zygotic nucleus. The production of haploid nuclei from diploid cells involved a process called meiosis. The immediate products of meiosis in plants are the spores. In plants microgenesis yields microspores and megasporogenesis yields megaspores. In terms of sexual differences, microspores furnish the male

gametic nucleus whereas megaspores supply the females gametic nucleus. Meiosis or reduction division gives rise to daughter cells that are not identical with the parent cell. It consists of two successive divisions (called I and II) of a nucleus accompanied by one division of its chromosomes. Each of the four daughter cells produced by one meiotic division contains only half of the number of chromosomes of the parental cells (the diploid, 2n generation). Meiosis is a compensatory reduction mechanism for the chromosome doubling that occurs through a cell fusion at fertilization in sexually reproducing organism. Without meiosis, the chromosome number would double at each fertilization, giving rise to bigger cells with greater chromosome numbers in each succeeding generation indefinitely (Fig.I-5).

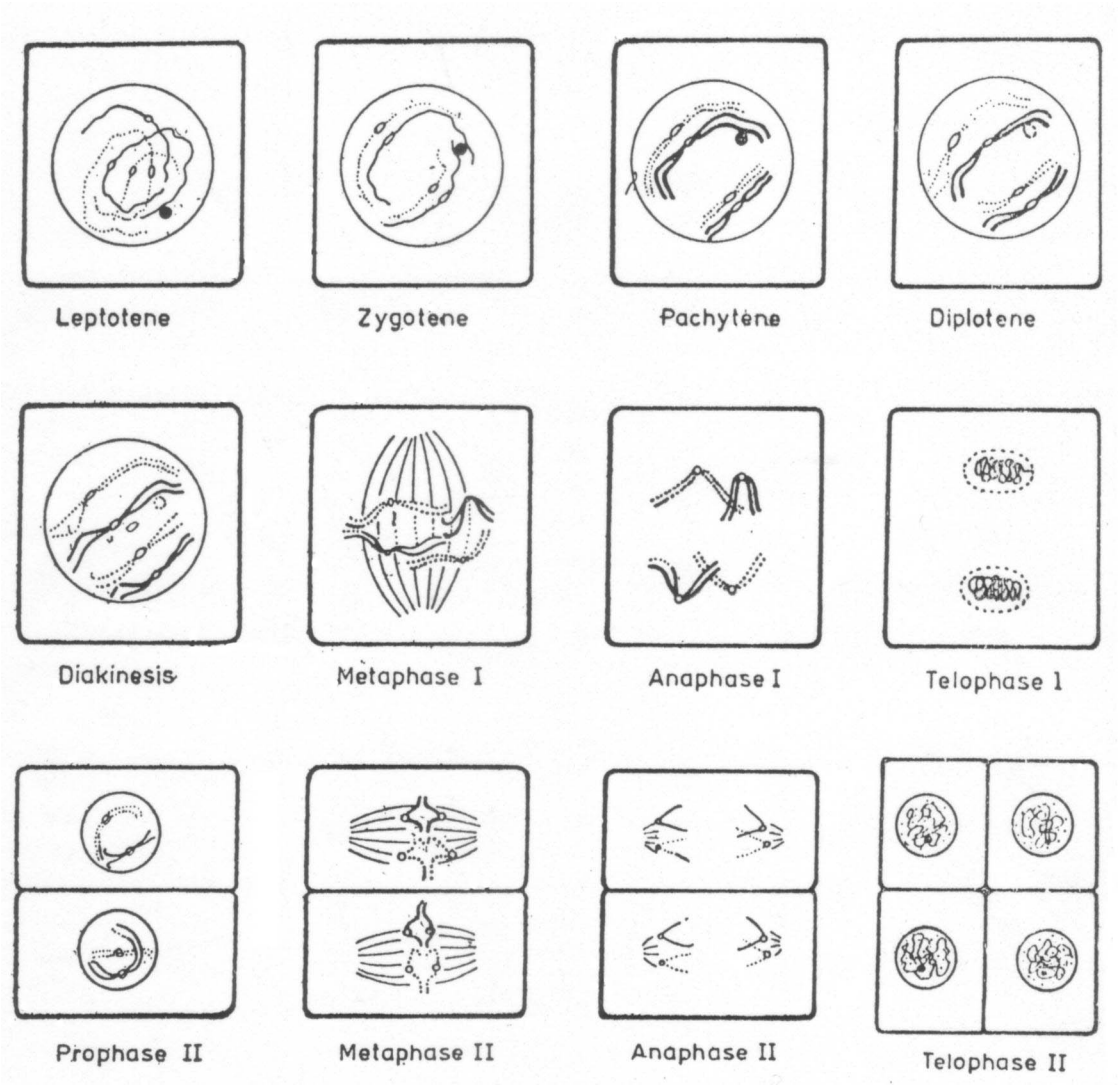


Fig. I-5 Diagrammatic representation of different stages of meiosis

PROPHASE I

It has a longer duration than the prophase of mitosis and has five separate stages. Meiocytes continue DNA synthesis beyond interphase into prophase and the RNA continues to be synthesized after completion of DNA duplication.

Leptotene - is the first generally accepted stage, the chromosomes are very thin and the homologue are randomly distributed and unpaired. There are many tiny swellings called chromomeres along the threads. Leptotene ends when the single threads pair or synapse with their homologues.

Zygotene - is marked by the lateral associations of the segments of two chromosomes. This pairing called synapsis, is the expression of the homology of the associated segments. Pairing continues throughout zygotene in a manner resembling a zipper.

Pachytene - at this substage, the synapsed chromosomes become shorter and thicker, and a highly organized structure of filaments called the synaptonemal complex between the paired chromosomes appears. The complex appears as parallel dense bands in a single plane curving and twisting along the axis between chromosomes.

Dipotene - The synapsed homologous chromosomes separate from each other except at the point where they are held together by the mutual switching of non-sister chromosomes at one or more sites along the chromosomes. This visual consequence of crossing over is called the chiasmata.

Diakinesis - is the end of diplotene where the associations of bivalent chromosomes are scattered throughout the nucleus. Terminalization occurs and the chiasmata moves away from the centromere and eventually slip off.

Prometaphase - the nucleolus disappears, the nuclear envelope breaks down, the spindle fibers forms and chromosomes are moved on the equatorial plane.

Metaphase I - the alignment of bivalents midway between the poles of the spindle apparatus characterizes the metaphase I. The centromere of each chromosome in the bivalents is associated with fibers from only one pole.

Anaphase I – the loss of all chiasmata for one or more bivalents marks the onset of anaphase I. Each group with the haploid number proceeds to its respective pole within the confines of the spindle occupying increasingly smaller volumes. The arms of each chromatid separate and each chromosome is obviously a dyad.

Telophase – each group of chromosome is enclosed within a nuclear membrane before the chromosomes reach the pole. The spindle apparatus disappears and nucleolus reappears and the chromosomes become longer and threadier.

Interkinesis – interval between the first and the second meiotic division where the primary meiocyte has produced either one secondary meiocyte with two haploid nuclei or two secondary meiocytes with haploid nucleus.

5.3 MEIOSIS II

The second meiotic division is essentially a mitotic division.

Prophase II – each nucleus enters prophase II at the same time and synchronously proceeds through the subsequent stages.

Metaphase II – the nuclear membrane disappears and the chromosomes are aligned in a plane midway betweent poles of the spindle apparatus. The centromeres of chromosomes at metaphase II are associated

with fibers from opposite poles.

Anaphase II - the separation of the chromatids at the centromere signals the onset of anaphase II. Each chomatid has a centromere and therefore is a chromosome at this time. Each group with the haploid chromosome number proceeds toward each respective pole.

Telophase II – each group of chromosomes is now enclosed within a nuclear membrane, the spindle apparatus disappears, the chromosomes become larger and threadier, and finally the chromatinic network of the interphase nucleus appears.

6. SPOROGENESIS AND FERTILIZATION

The four haploid nuclear products of microsporogenesis for the microsporocyte or pollen mother are packaged to give four viable uninucleate microspores. After the microspores separate, the microspore nucleus divides mitotically producing a generative nucleus and a tube nucleus. In some species, these cells are shed as pollen grains and after landing on the stigma germinate to produce a pollen tube which grows in the direction of the female gametophytes in the ovary. The generative nucleus proceeds down the pollen tube and divides mitotically to produce the two haploid sperm nuclei. In most higher plants, each haploid product of megasporogenesis is enclosed within a megaspore. The three of the four megaposres forming a linear tetrad degenerate and the basal megaspore develops into the female gametophyte, or embryo sac. The pollen tube of the male gametophyte delivers the two sperm nuclei to the embryo sac where one nucleus fertilizes the egg and produces the diploid nucleus (Fig. I-6a, 6b).

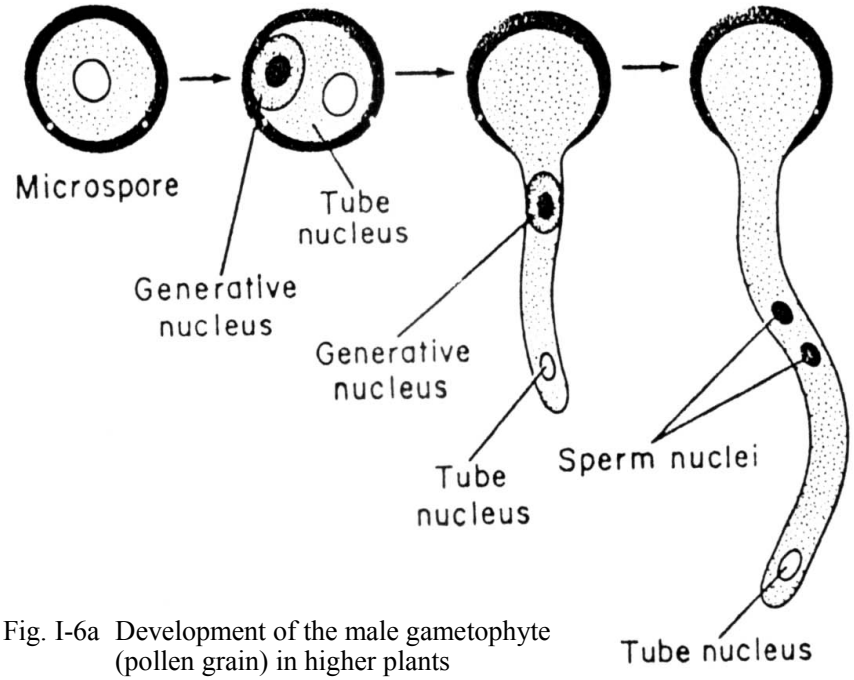


Fig. I-6a Development of the male gametophyte (pollen grain) in higher plants

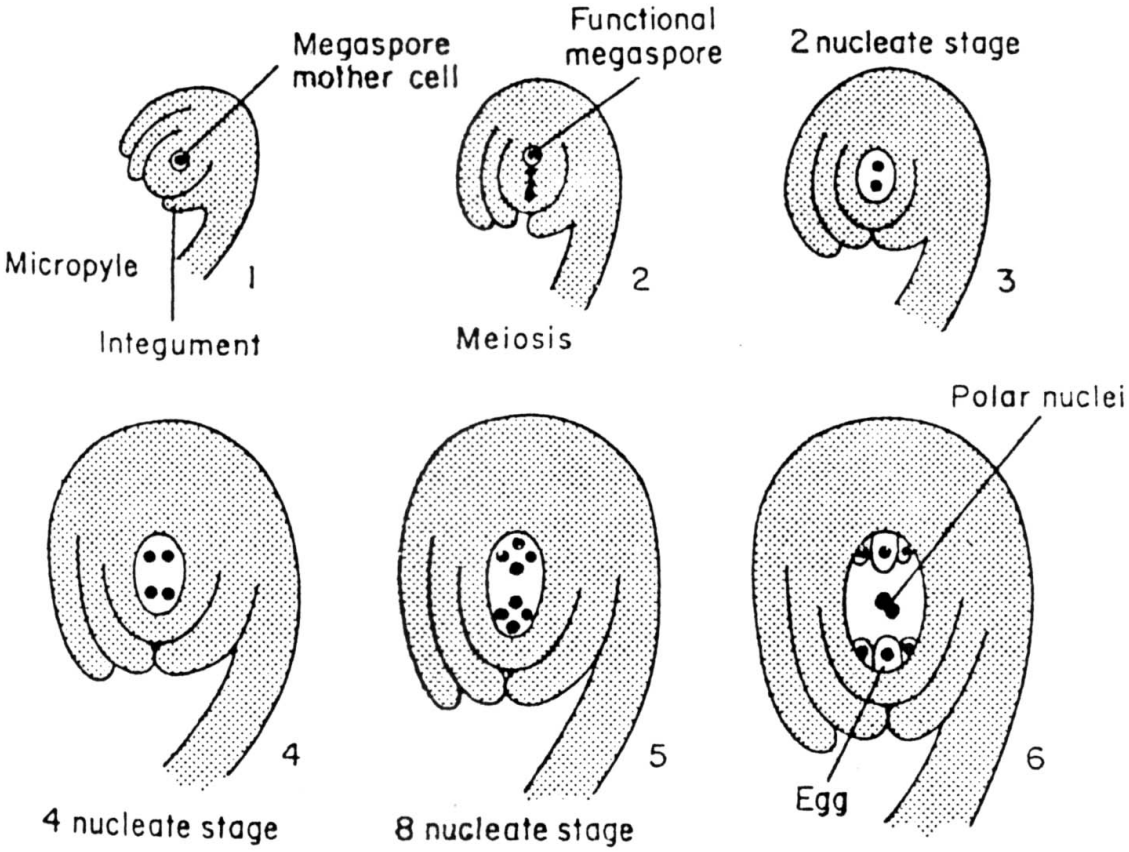


Fig. I-6b The basic pattern of the development of the female gametophyte (embryo sac) in higher plants

7 EFFECTS OF IONIZING RADIATION

Radiations both ionizing and those of lesser energy (ultra violet light) are physical mutagens that are quite efficient at producing changes in genetic material. Its production if mutations is its most important biologic effect. Nonif its other effects is so far reaching in consequences both for the individuals in which mutation occurs (whether single cell or multicellular organism) and for the population of which a mutant bearing individual is a member. Radiation as a mutagen is important because: 1) it is the most effective one known for producing mutations in quantity; 2) it is almost an indispensable tool for geneticists as its affords an opportunity to study the very nature of the gene; 3) mutational frequency is increased so that changes are produced in genes at will; 4) changes that are made give an important clue as to what has been changed; 5) by producing mutations in abundance radiation facilitates the study of consequences of genetic mutation in individuals (cells or organisms) and in population in which these mutations arise.

7-1 RADIATION EFFECTS ON DNA

In the production of mutations, radiation acts by ionizing nitrogenous bases in the DNA chains specifically at DNA synthesis. Base-change or base-deletion gives change in critical base sequence of the molecules. Ionization of one or more of the bases with free radicals that radiation produces may alter base structure that they no longer possess the characteristics which they had before exposure to radiation.

7-2 RADIATION EFFECTS ON CHROMOSOME STRUCTURE

Ionizing radiation can, either by direct or indirect action, break chromosomes. Structural rearrangements of chromosome and even loss a part of chromosome from cells as well as the whole chromosomes may follow a radiation induced break. Loss of chromosomes or chromosome fragments produces distinct deviations from the cellular status quo. Even the loss of very small pieces of chromosomes gives damage to the cells in which it happens, and the loss of large chromosomes is serious enough to bring about cell death within a few cell generations.

The type of chromosome structural change induced by ionizing radiation have been well documented and detailed descriptions and illustrations of those seen to occur in irradiated samples may be found in many publications.

There are three basic categories of "break", a) the chromosome type where lesions involved are seen at metaphase to affect both chromatids of a chromosome at the same locus (balanced types) corresponding to chromosomes unsplit at the time of irradiation; b) the chromatid type of aberration where the lesions involved affect only one chromatid at any particular locus (unbalanced types) corresponding to chromosomes which had split longitudinally before irradiation and sub chromatid type of aberration where the lesions involved affect only one chromatid at a very minute locus, corresponding to chromosomes exposed at prophase and may affect only one chromatid at sub-chromatid locus.

A. CHROMOSOME-TYPE ABERRATIONS

1. Exchange aberrations involve the interaction of two or more lesions.
2. Interchange-when lesions occur in the arms of different (homologous or non-homologous) chromosomes
3. Interchange-when lesions are small and all within the same chromosome.

a. Inter-arm interchanges when the lesions are in opposite arms with respect to the centromere.
b. Intra-arm interchanges when both lesions are in the same arm.

B. CHROMATID TYPE ABERRATIONS

1. Exchange-similar to description above
2. Interchange
3. Intra-change
    a. Interchromatid- when the interacting lesions are in different (sister) chromatids
    b. Intra-chromatid-when both lesions are in only one of the sister chromatids.

7.3 EFFECTS OF RADIATION ON MITOTIC APPARATUS

Ionizing radiation interferes with the normal orderly process of cell division. This interference with cell division is the most immediate conspicuous effect of ionizing radiation on the transmission of the genetic material. All ionizing radiations as well as ultra-violet radiations and other mutagens inhibits mitosis. All mitoses are not stopped, cells in mitosis as far along as mid or late prophase when radiation is given, may complete their divisions. But those in interphase and those about to enter prophase, will be prevented from doing so and their divisions will be inhibited. Those in the early stages of prophase may not complete division but instead regress, returning to the interphase condition. When inhibition is over, normal cells would begin to enter the mitotic cycle to divide, hence a profusion of mitoses following a period of inhibition, i.e. synchronization of mitoses in irradiated group of cells or tissue. The inhibitory effect is dependent upon dose but is transitory. Radiation also brings about non-disjunction of centromeres at metaphase and the unequal distribution of chromosomes to daughter cells at anaphase. Ionizing radiation in addition causes lagging of chromosomes on the spindle at anaphase, an effect ascribed to changes brought about in the centromere. Ionizing radiation promotes cross-over in the region of the centromere due to an effect of radiation on the centromere.

8. PLANT BIOTECHNOLOGY (*IN VITRO* CULTURE)

A wide variety of technologies in tissue culture, genetic manipulation and molecular biology have been developed in a number of plant species. These laboratory-based technologies are now jointly referred to as plant biotechnology. Plant biotechnology had direct application in mutation breeding, genetic improvement and production of plants. The fundamental basis for the application of plant biotechnology is the capacity of plant cells and organs to develop into complete plants which can be grown to maturity.

1. Axillary buds. One of the most exciting and important aspects of *in vitro* cell and tissue culture is the capability to regenerate and propagate plants from cultured cells and tissues. The simplest type of in-vitro plant propagation is the stimulation of axillary bud development. The technique exploits the normal ontogenetic route for branch development by lateral (axillary) meristems. The buds are treated with hormones to break dormancy and produce shoot branches. The shoots are then separated and rooted to produce plants. The shoots are also used as propagules for further propagation. Many ornamental plants and woody species are propagated commercially by axillary bud proliferation. Axillary bud proliferation results in an average tenfold increase in shoot number per monthly culture passage, that in 6 months it is possible to obtain as many as 1,000,000 propagules or plants from a single explant.

2. Adventitious shoot and organogenesis. The technique for adventitious shoot organogenesis from leaf sections covers several defined steps in a typical micropropagation system. The first step is the initiation of a sterile culture of the explant (stage I). The second step is the multiplication of shoots or other propagules from the explant (stage II). Adventitious shoot proliferation is the most frequently used multiplication technique in micropropagation systems. The culture media and growth conditions used in stage II systems are optimized for maximum rates of multiplication. The third step is the development of roots on the shoots to produce plantlets (stage III). Specialized media may or may not be required to induce roots depending on the species. The final step is to produce self-sufficient plants (stage IV) which usually involves a hardening-off process and acclimatization to growing in soil mixes under greenhouse conditions for later transplanting to the field.

3. De novo organogenesis. The first step in plant regeneration by organogenesis for callus and cell suspension or de novo regeneration is to establish callus or cell suspension cultures. Explant tissues generally show distinct planes of cell division, various specializations of cells, and organization into specialized structures such as the vascular system. Callus formation from explant tissues involves the development of more random planes of cell division. There are five phases in callus growth:

a. A lag phase, where cells prepare to divide.
b. An exponential phase, where cell division is highest
c. A linear phase, where cell division slows but the rate of cell expansion increases
d. A deceleration phase, when rates of cell division and elongation decrease, and
e. A stationery phase, where the number and size of cells remain constant.

Cell suspensions are initiated by transfer of callus pieces into flask with a liquid medium placed on a gyratory shaker to provide aeration to the cells. As new cells are formed they are dispersed into liquid medium and become clusters and aggregates. Cell suspension can be maintained by subculturing in flask called batch cultures. Cells from suspension cultures can be harvested by filtration or transferred to agar medium and grown as callus.

4. Somatic embryogenesis from de novo. In somatic embryogenesis, somatic cells develop by division to form complete embryos similar to zygotic embryos. The bipolar structure of the somatic embryo contains both shoots and root meristems. As the embryos develop, they progress through distinct structural steps i.e. the globular, heart torpedo, cotyledonary and mature stages.

5. Direct somatic embryogenesis. This is the formation of somatic embryos or embryogenic tissue directly from explant without the formation of an intermediate callus phase. To induce direct embryogenesis, explant most often used is the immature zygotic embryo. This tissue is already embryogenic in nature and apparently requires less nurturing.

9. DOSE DETERMINATION OF RADIATIONS

Several methods are used to determine the dose of radiations. Among the commonly used are:

1. Ionization Chamber

These are chambers whose walls and gas consist of materials that have been elemental composition similar to the body tissue. These are called tissue ionization chambers. In mixed radiation field, two chambers are used, one sensitive to both fast neutrons and gamma radiation and the other insensitive to neutrons.

2. Fricke Dosimeter

This method is useful for gamma dose measurement with dose ranging from 400 – 40,000 rads. The standard Fricke solution is composed of the following:

$Fe(NH_4)$ 2.6 H_2O ---------- 10 mol/ l
Sulfuric chloride, NaCl ---------- 10 mol/l
Sulphuric Acid, H_2SO_4 ---------- 0.4 mol/l

The absorbed dose D is obtained from the formula

$$D = \frac{9.64 \times 10 (d-d)}{G(Fe^{+3}) \cdot p.l.e.}$$

Where : d = optical absorption of irradiated solution at 304 nm
d = absorption of unirradiated solution (control)
$G(Fe^{+3})$ = G-value 100 eV -1
= 15.5 for ^{60}Co radiation
p = density of the solution
l = thickness of solution (normally 1 cm quartz cell are used)
e = extinction coefficient of Fe^{+3} (1/mol cm)
= 2190 at 304 nm and 25 ^oC
(e increases with temperature by 0.7% per 1 ^oC

(*Prepared by: Mr. A.B.Asencion, PNRI)

3. FeCu Dosimeter

This method is used when gamma and fast neutron doses from mixed field are to be determined separately. Usually two chemical systems with different yields are used.

4. Fast Neutron Dose Estimation by Sulfur Activation

This method make use of the ^{32}S (n,p) ^{32}P reaction to estimate the fast neutron fluence and fast neutron dose. Since the actual neutron spectrum is not known, results of measurement are given in terms of equivalent tissue fluence:

$$O_f = \frac{As \cdot T (n/cm^2)}{(6\,f) \cdot n}$$

Where : As = specific saturation activity (dps/g)
(6 f) = average cross-section of the reaction for a fission neutron spectrum
$= 65 \times 10^{-27} (cm^2)$
T = irradiation time (sec)
N = number of ^{32}S atoms/ g sulphur
$= 1.791 \times 10^{23}$

10. TREATMENT METHODS

10.1 RADIATION TREATMENT

There are several methods in treating materials for irradiation. Among them are acute, chronic, recurrent and combined.

Acute treatment is done in a few minutes or a few hours. Usually the dose is at the optimum level and applied only once.

Chronic treatments are applied over long periods of time. The dose is small, fractionated and delivered in minutes or few hours.

Recurrent treatment on the other hand is carried out on materials that had been irradiated already in the previous generation

Based on the results obtained from several experiments, the beneficial effects come mainly from acute and chronic treatments. The benefits derived from the recurrent treatment appear not to compensate for the extra labor and time in handling materials.

Any plant part can be used for mutagenic treatment and the most commonly used ones are seeds, pollen, whole plant, cuttings, tuber, corm, bulbs, stolons and cultured cells, tissue and organs. Seeds can be easily irradiated using X-ray machines or gamma sources in a greenhouse or shielded room or gamma cells. The advantage of using seeds is that they can be irradiated in many physical environments. They can be dessicated, soaked, heated or frozen. They can be maintained for a long period of time. This is advantageous because under dry conditions, no significant damage can affect the seeds. Also under this condition, it is favorable since the factors that modify the radiation damage are controlled. One very important factor however is the control of the moisture content. It should be maintained between the 12 – 14 %. This is accomplished by putting the seeds in mesh bags and storing them in a dessicator with 60 to 70% (by volume) glycerol solution for 4 days. This is important to control damage to the seeds. At high water moisture content the molecule is changed when exposed to radiation. The following results can be seen when free→ radicals recombine after irradiation.

E – aq + e-aq	--------→	H_2 + 2 H_2O
H + H	--------→	H_2
OH + OH	--------→	2 H_2O_2

All the radicals are highly reactive thus resulting in chemical changes that are similar to the effect after direct ionization of the molecule.

After the moisture is stabilized the seeds are pasted in appropriate cardboard with stand so that the seeds are perpendicular to the radiation source during irradiation. This is to make the treatment even particularly when it is for radiosensitivity studies. Irradiation should be done as soon as the set up is completed. For mutation breeding purposes, the seeds can be placed inside a small coin envelope or plastic bag and attached to a card board so that during irradiation it is perpendicular also to the source. As soon as the seeds are irradiated, they should be packed in a plastic bag and stored in a refrigerator if sowing cannot be done immediately. The doses found appropriate for cereals and other seed crops ranges from 100 – 600 Gy.

For cuttings and other asexually propagated crops, care should be made not to expose the root system. Placing lead blocks in front of the base during irradiation is sufficient to protect the root system from being irradiated. The dose range found to be appropriate including cultured cells between 5 – 6 Gy.

WHOLE PLANT

For the whole plant, they can be irradiated in gamma field, gamma room or "Greenhouse" with lighting and water provisions to maintain the plants especially when the method of treatment is chronic and lasting for a number of weeks or more. Seedlings and small plants can be irradiated by same way.

POLLEN GRAINS

Pollen grain is not as convenient as in seed irradiation. In the first place, collecting pollen is a problem and sometimes not enough can be collected and viability is not long in some cases. Besides special provision is needed for chronic irradiation. So one should work fast when it comes to irradiating pollen. One advantage however, is that pollen rarely produce chimera.

Pollen can be irradiated using U.V. machine, X-ray machine, gamma cell, or ion beams. In cases of potted plants that are flowering, gamma garden or gamma field is ideal. In the case of cereals like rice, panicles can be collected in the early hours, irradiated, wrapped in moist cloth and then used in pollination at 10 – 12 a.m. where the stigma is receptive.

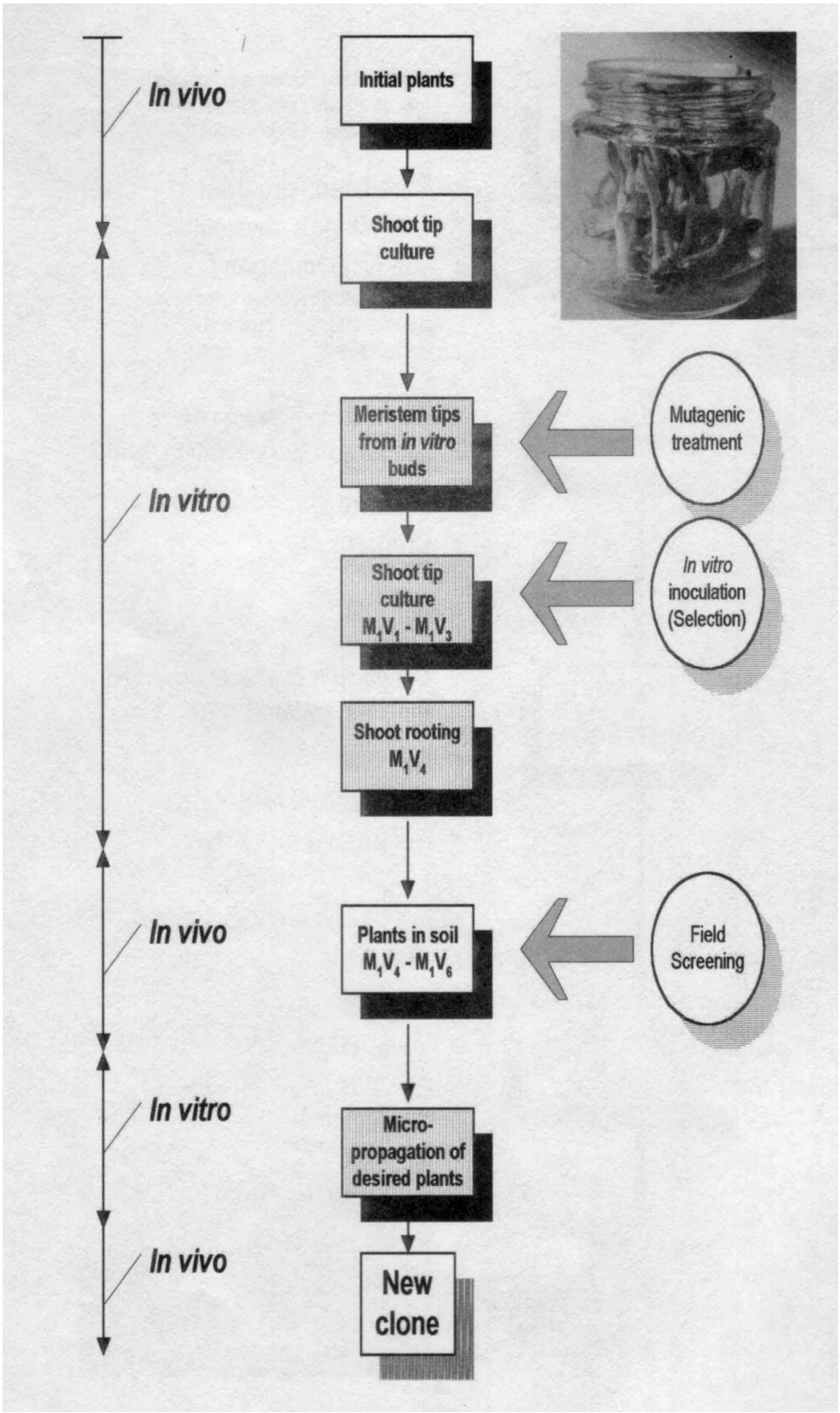
CULTURED CELLS AND TISSUES

With the popularity of biotechnology, irradiation of cultured cells and tissues will become very common in biological studies (Fig. I-7). The advantage of treating single somatic cells is that large numbers of homogenous individuals are available in small area. Another is that mutant characteristics are manifested almost immediately because of the limited metabolic product in the cell. Still another advantage is that a single mutated character is not masked by a dominant allele. Selection can also be made efficient and specific since the cells are cultured according to the biological need to isolate mutants from a large mix cells. Furthermore, selected single haploid can be regenerated and diplodized for breeding purposes or genetic studies. This makes the operation less costly.

MERISTEM

Whether one deals with seeds or any plant part in mutagenic treatment, one deals with meristem. In the case of seeds, the resting embryo is the target and it is composed of a few primordial leaves, a number of axillary buds and the apical meristem. In the case of cuttings, one is dealing with the shoots that developed from a single epidermal cell of the petiole. Thus treating with mutagens will in almost homohistant or solid mutants instead of chimeric plants. Chimera, however, can be observed especially in merstem. Three types of chimera are mericlinal, periclinal and sectoral.

DEVELOPMENT OF CHIMERA (Fig. I-8, 9a, 9b)


In vivo
Initial plants
Shoot tip culture
In vitro
Meristem tips from in vitro buds
Mutagenic treatment
Shoot tip culture M1V1 - M1V3
In vitro inoculation (Selection)
Shoot rooting M1V4
In vivo
Plants in soil M1V4 - M1V6
Field Screening
In vitro
Micro-propagation of desired plants
In vivo
New clone

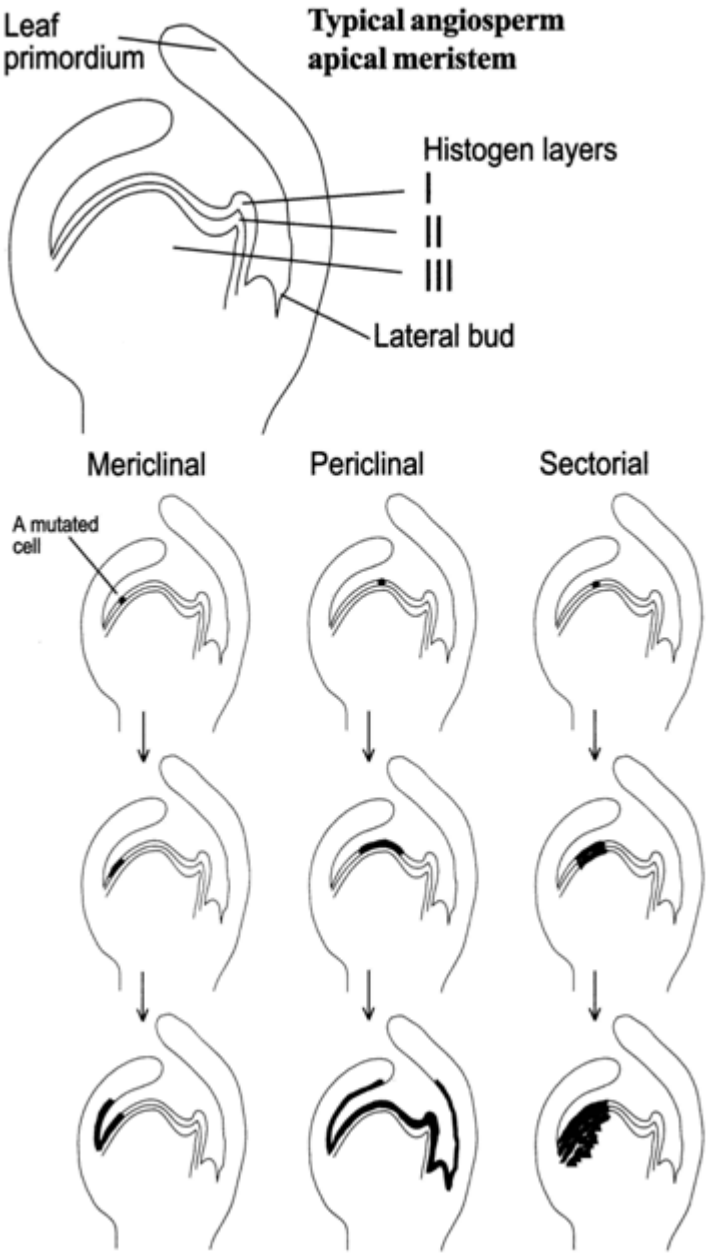


Fig. I-8 Development of Chimeras in Shoot tips (apical meristems)

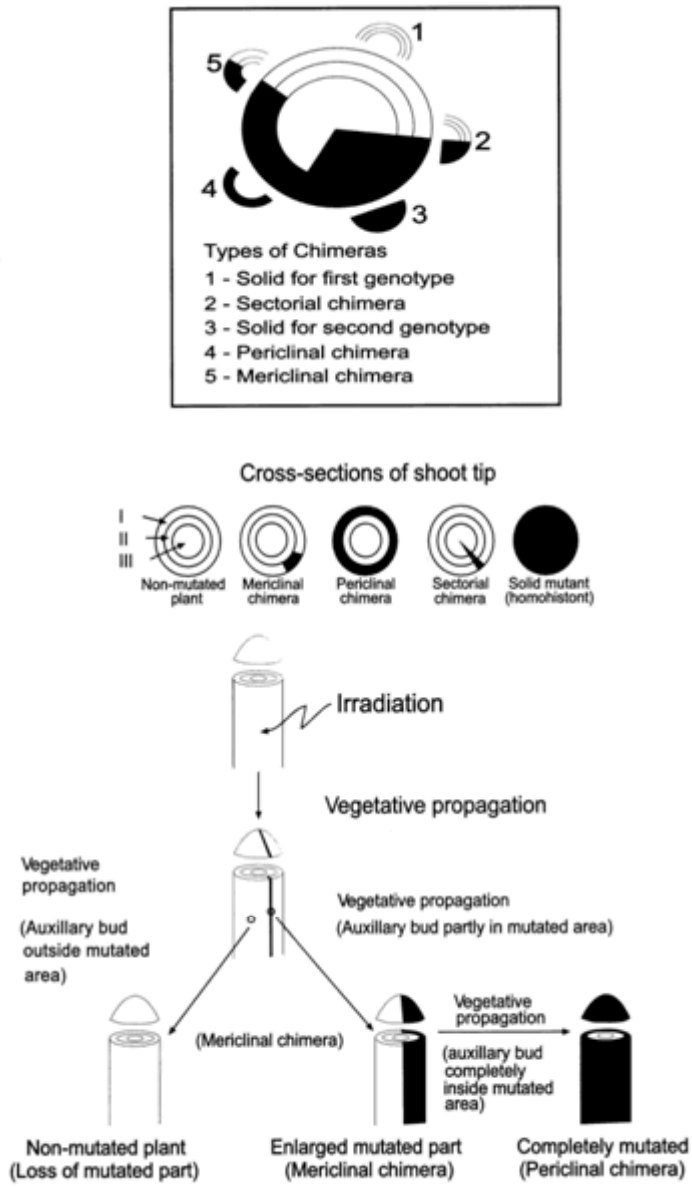


Fig. I-9a Types of chimeras and vegetative propagation of mutated sector

Source: Research Highlights on: The Use of Induced Mutations for Plant Improvement
in Malaysia. National Committee on the Use of Induced Mutations in Plant breeding
Malaysian Institute for Nuclear Technology Research (MINT) Bangi, 1998

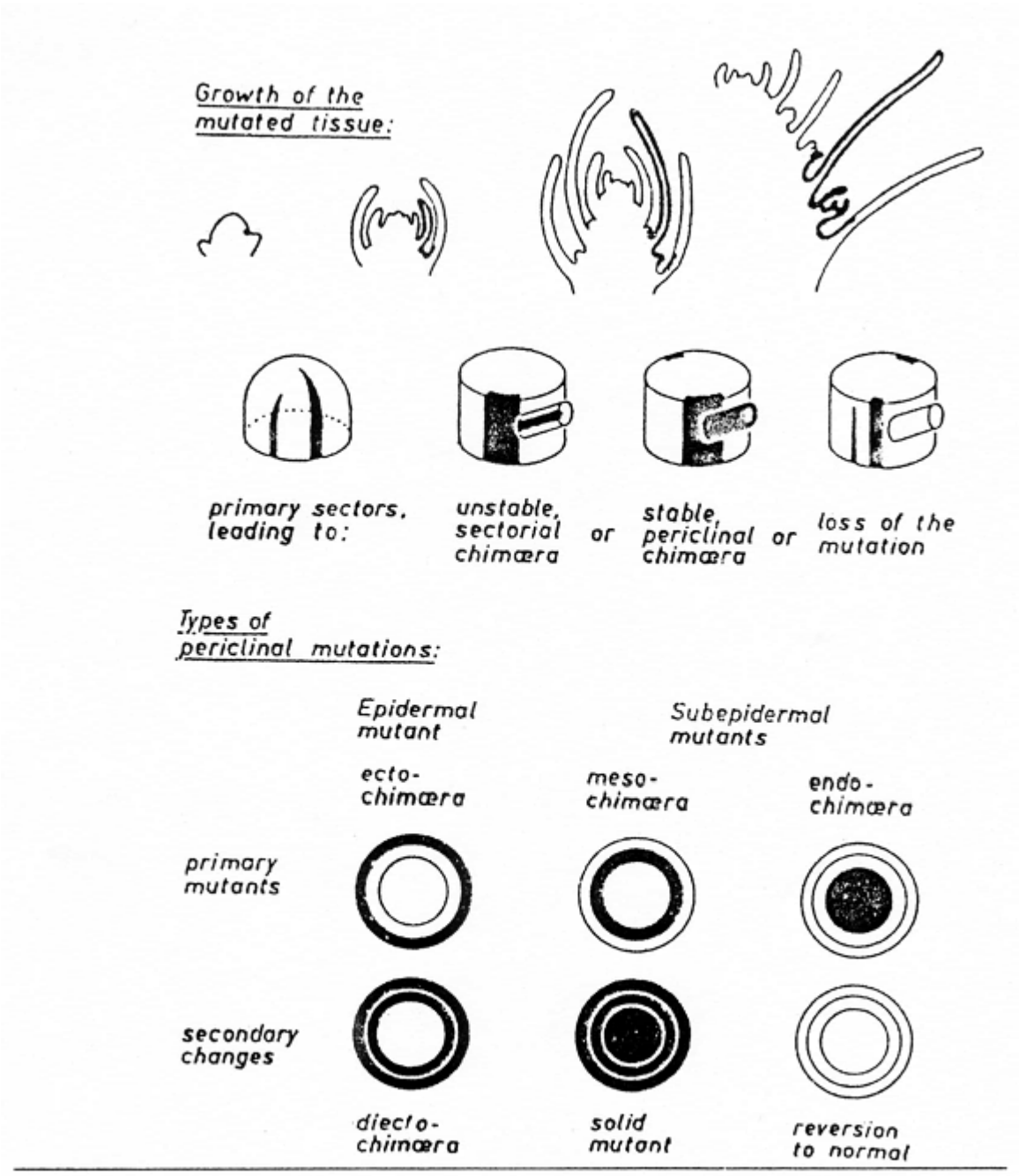


Fig. I-9b Schematic presentation of mode of origin and chimaerical structure of somatic mutations

10.2 CHEMICAL TREATMENT

The use of chemicals to induce mutation vary widely but most of the treatments involves seeds rather than other parts of the plants. The reasons for this is that chemicals are not readily absorbed to plant tissues as by seeds. Techniques however, to overcome this problem were developed and one of these is by placing a cotton on the part to be mutated and the mutagen is put on the cotton cot it can be retained for sometime.

Only two examples of treatment procedures will be highlighted here for chemical treatment since the methodology is more or less representative of the technique.

ETHYL METHANE SULPHONATE (EMS) TREATMENT FOR SEEDS

Prepare seeds with more or less 95% germination and place them in mesh bags. About 200 seeds per bag would be enough so that the seeds will be in one layer. Stabilize the moisture content of the seeds to between 12-14% by putting them in dessicator with 60% glycerol solution for 4 days.

Prepare water bath to stabilize the temperature during treatment with mutagen. Pre-soak the seeds in distilled water for up to 16-20 hours at room temperature (20-22ºC).

One half hour or a few minutes before the end of presoaking time, prepare the mutagen. At least 1 ml per seed should be made so that prepare 1 liter if the number of seeds to be treated is 1000. Use distilled water. Usually it is recommended to use mole in preparing the solution although the percentage is sometimes used. For EMS, the molecular weight is 124.2, so to prepare for 1 mole simply weigh 124.2 g. of EMS and dissolve it in distilled water to make a final volume of 1000 ml. Based on this, one can adjust his/her requirement by ratio and proportion.

Soak the seeds for two hours at constant temperature of 30-35ºC. When temperature rises, add ice until the desired temperature is maintained.

Remove the seeds after soaking and wash them in running tap water for at least 4 hours.

The seeds can be planted immediately after a short drying under an electric fan. If the seeds cannot be planted right away, they should be dried back to 13% moisture content and stored in a refrigerator to retain their viability.

For rice seeds, it is advisable to de-hull them before any chemical mutagenic treatment. A word of caution when doing the treatment. Do it always inside a fumehood.

SODIUM AZIDE (NAM_3) TREATMENT FOR SEEDS

Basically the preparation is the same as that in EMS except that in sodium azide, pH has to be adjusted to 3 during treatment because it has been found that this mutagen is effective only under acidic condition. After stabilizing the moisture content of the seeds, the seeds are pre-soaked in free flowing water for 2-16 hours at 20-23ºC with continuous bubbling of the water.

Stock solution of 1 M should be prepared ahead using distilled water. The stock solution can be stored in a refrigerator for long period of time without losing its potency. Sodium azide has a molecular weight of 65 so that to prepare 1 M stock solution, simply weight 65 g of the NaM_3 crystals and dissolve in distilled water to make a final volume of 1000 ml using a volumetric flask.

Since sodium azide requires low pH, a buffer of 0.1 M is needed and should be prepared one half hour before the treatment using mono basic potassium phosphate, the molecular weight of which is 136.09. Thus, to prepare 1 M, weigh 136.09 g of the crystals and dissolve in distilled water in a volumetric flask to make a final volume of 1000 ml. The pH can be adjusted to 3 by adding drop by drop concentrated phosphoric acid using pipette, reading the pH of the solution with an appropriate pH meter.

To prepare the 10^{-3} M sodium azide solution, add 1 ml of the 1M sodium azide to 999 ml phosphate buffer (pH 3) to make a final volume of 1000 ml. One ml of this solution must be used for each seed so that 5,000 ml should be prepared for 5,000 seeds.

Put the pre-soaked seeds in the solution for 2-4 hours with the temperature of the solution maintained at 20ºC and continuously bubbled.

After the treatment, the seeds are washed in cold flowing water for two hours. The seeds can be planted immediately after short drying period. Alternatively, the seeds can be dried back to 13% and stored in a refrigerator if they cannot be planted immediately to maintain their viability.

11. HANDLING OF THE TREATED MATERIALS AND THEIR SUCCEEDING GENERATIONS

Schematic mutation breeding for seed crop plants is shown in Fig. 10.

When seeds are exposed to ionizing radiation or chemical mutagen, several effects can be observed. Measure and analyze the physical damage and chromosomal aberrations. The physical damage can be determined by cytological analysis and by measuring plant injury in the M_1 generation. Plant injury can be measured by taking :

Seedling height (determined 10-14 days after)

Root length (determined soon after germination in the laboratory)

Emergence under field or laboratory condition

Number of spikes per plant

Number of florets per spike

Number of seeds per spike

Fruits and/or seeds per plant

Determination of M_1 injury using seedling height and survival should be a routine procedure in mutation breeding, because it has been established that these characters are correlated with M_1 mutation frequency.

There are three methods in raising seedlings to determine seedling height, namely, Flat Method, Petri-Dish Method and Growing-Rack Method.

In the Flat Method, the seeds are sown in boxes filled with a sterile mixture of sand and soil (50:50) or any convenient medium. The cheapest way of sterilization can be done by firing the media in a suitable container for at least one hour with complete stirring from time to time or it can be accomplished using chemicals. The sterilized mixture is placed in seed boxes and covered with plastic film and can be used for planting the following day. The treatments are arranged in a Randomized Complete Block Design (RCBD) and replicated at least 4 times. Randomization can be accomplished by using random table or by drawing lots. The seedling height is measured on the first leaf when the growth stop already(10-14 days after).

The second is the Petri-Dish Method. This is done by putting wet blotting paper in the petri-dish and the seeds are sown on them. The seedling height and root length are determined 7 days after.

The third is the Growing-Rack Method. In this method, the seeds are placed between two wet blotters and supported vertically by wire or PVC rack. The racks are placed in aluminum, plastic or wooden trays lined with plastic and water is added to the tray making sure that the lower edge of the filter paper is immersed. The racks are placed in a controlled uniform environment either in a "Greenhouse" or a chamber.

Measurement of the seedlings height and root length can be made 7 days.

For the other data, gathering can be done in the field or "Greenhouse". Planting for this purpose should need more plants (1000-5000) and spacing should be closer to each other to minimize tillering especially

for cereals. The seeds gathered from this (M_1) planting can be used also for scoring chlorophyll and morphological mutations in the M_2. Sometimes for critical experiment, it is necessary to bag the plant individually to prevent out-crossing. This will make the result more reliable.

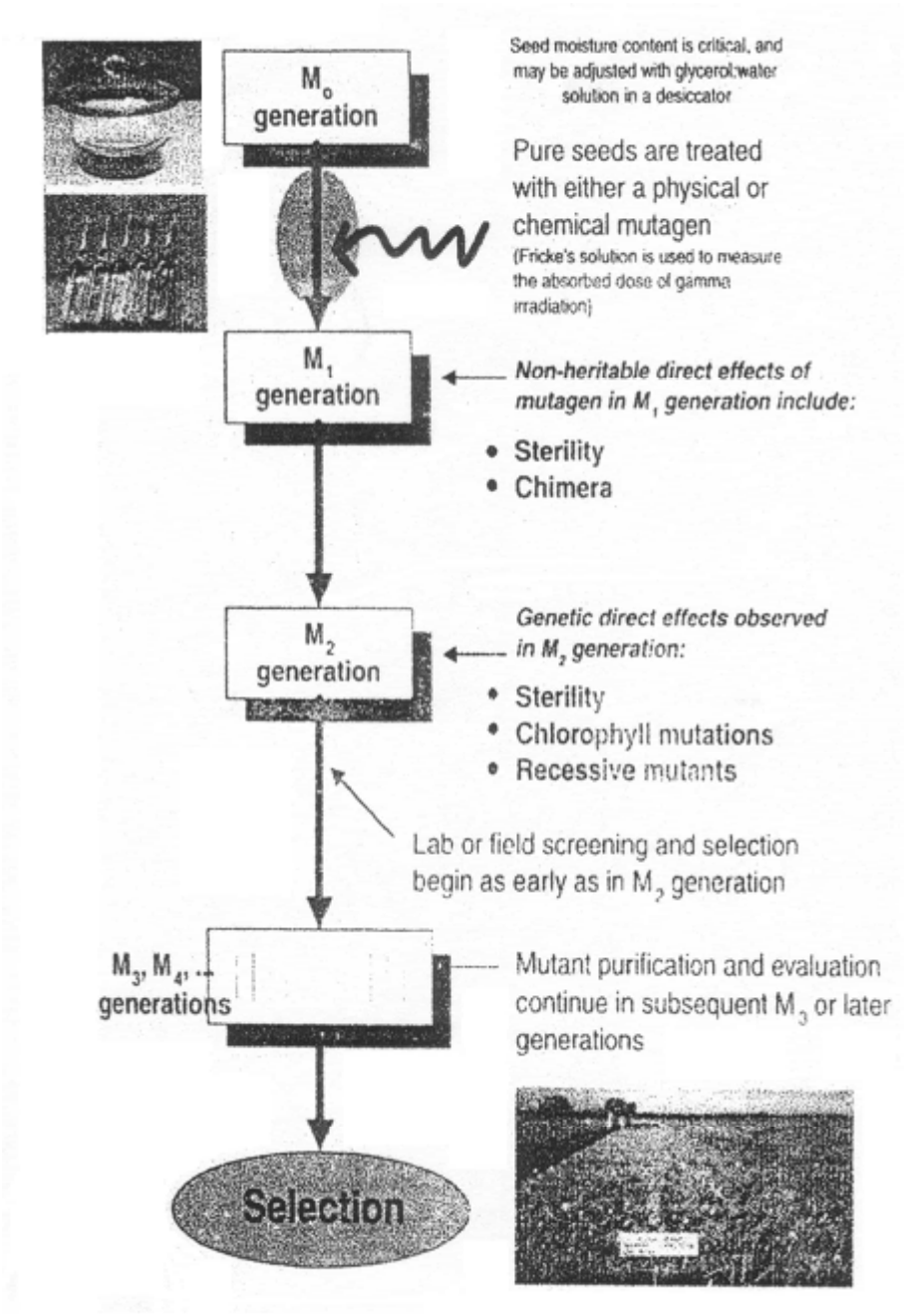


Fig.I-10 Schematic mutation breeding for seeded crop plants

Source: Research Highlights on: The Use of Induced Mutations for Plant Improvement in Malaysia, National Committee on the Use of Induced Mutations in Plant Breeding. Malaysian Institute for Nuclear Technology Research (MINT) Bangi 1998

11.1 CHLOROPHYLL AND MORPHOLOGICAL MUTATION IN THE M_2 GENERATION

Two methods can be used in scoring chlorophyll mutation in the M_2. One method is based on 1000 M_2 plants or seedlings and the other is based on the M_1 plant and M_2 seedlings. The M_1 plant method make use of the number of M_1 plants sown and the number of plants that have mutations. The M_2seedling method is done by counting the total number of mutants and also the total number of seedlings. The types of chlorophyll and morphological mutations should be separately counted and computed based on the total number of seedlings. The data can be tabulated and entered in the appropriate Tables below. (Table I-1-5)

Table I-1. Frequency of Chlorophyll Mutation

Treatment	No. Studied M1 Plt. M_2 Seedling	No. of M_1 Plant Segregating	No of M_2 Mutant Seedling	%Chlorophyll M_1 Plants	%Chlorophyll M_2 Seedling

Table I-2. Frequency of Chlorophyll Mutation Types in M_2 Seedling

Treatment	Total M_2 Seedling		Types of Seedling Mutation					
			Virescent		Chlorina		Xantha	Albina
No. %	No.	%	No.	%	No.	%		

Table II-3. Mutation Spectrum in M_2

Treatment	Total Mutant Seedlings	Chlorophyll Mutation			
		Viable Type		Lethal Type	
		No	%	No	%

Table I-4. Frequency of Morphological Mutations

Treatment	No. Studied		No. of M_1 Plant Segregating	No. of M_2 Mature Plts.	% Mutation	
	M_1 Plants	M_2 mature			M_1Plts.	M_2Plts.

Table I-5. M_2 Morphological Mutation

Treatment	No. of Mutant Plants	Characteristics of Mutant

Handling of M_2 and Succeeding segregating Population

Several methods are employed to handle the M_2 and succeeding segregating population (Fig. I-10 Among them are the bulk method and pedigree methods. Sometimes the two are combined.)

11.2 BULK METHOD

Bulk of M_1 population - the bulk M_1 seeds are planted and observed for variability. This method is efficient if the mutated character is easily identifiable. This method is also efficient in the case of mechanize screening for size of seeds, weight and shape.

a. M_1 Population bulk ------ M_2 single seed bulk --------- M_3 ear to row progenies
b. M_1 Population bulk ------ M_2 ear to row progenies (this is a combination of Bulk and Pedigree)

11.3 PEDIGREE METHODS

a. M_1 ear to row bulk - The M_1 ear in this method is randomly selected and small number of progenies are needed.

b. M_1 single-seed or multiple seed bulk - In this method a single seed is selected from each plant and constituted as M_2 population. This is followed by a single plant progeny test in M_3 or single M_2 ear for progeny selection in the M_3. This is the most efficient, low cost and space-wise method but requires the competency of the Plant Breeder in identifying the mutants.

c. M_1 plant to row - This method utilizes all the seeds from a single plant. However, about 50 or so seeds is enough to plant in each pedigree row. The advantage of this method is that one can note the specific origin of the mutant-lines. The disadvantage is that one will need a bigger space to plant the segregating population and therefore a little costly.

d. M_1 ear branch, pod, fruit (within plant) to row- This method is the most precise when it comes to identifying the origin of the mutant but is also the most expensive.

11.4 HOW THE MUTANTS ARE SELECTED

For the trained eye of the breeders, visual selection of the mutants phenotypic variation is the most effective. Depending on the breeder's objectives, mutants that meet them are selected disregarding the seemingly worthless mutants. Since the value of the other mutants cannot be ascertained immediately, it is worthwhile to select and preserve them for future studies and possible utilization. For example in dwarf rice mutants, their worth in breeding cannot be fully appreciated unless the genes controlling them are studied and compared to already established genes like sd_1, sd_4, sd_6 etc. This is to preclude uniformity of source of dwarfing genes in the released varieties which may prove disastrous if the varieties succumb to a particular disease or pest.

Visual selection is effective in identifying earliness, plant height, color changes, non-shattering, adaptation to soil, climate, day length, resistance to pests and diseases etc.

Another method of selection is mechanical or physical method. This method is very effective in handling large quantities of M_2 seeds particularly for sieving size, shape, weight, density etc.

Chemical, biochemical, physiological, physio-chemical and other screening procedures may also be needed for selecting certain kind of mutants but almost all employ visual observation including the much newer biotechnology techniques like PCR and related technologies.

Selection usually start from the M_2 and confirm in the M_3. Since it is not sure for the mutant to be homogenous already in all characters in the M_2, selection is necessary even in the M_3 generation. This is so because of the epistatic interaction among genes. In some cases, it may be necessary to reselect even in an M_3 line and grow the M_4 particularly in polyploids.

11.5 HOW TO DETERMINE WHETHER A VARIANT IS A MUTANT OR CONTAMINANT

A variant can be considered a mutant if the frequency of appearance is very low even in a bulk M_2. It is also a mutant if it appears as one to a few individuals in branch, spike or plant progenies and never appearing in a 3:1 ratio (but lower) in more than one or few of several branches or spike or progenies. In addition, the variant can be considered a mutant if similar variants have been isolated from several different progenies in a treated population at a much higher frequency than that obtained in the control.

Aside from the above criteria, selected variants should breed true already in M_3 if not in the M_2 and the variation should be limited to one or few traits.

When the variants back-crossed to the parent variety and to some other strains should show a simple genetic segregation.

Phenotypically, the mutants should show similarity to the parent variety except for the modification

involved. Even in complex changes in phenotype, similarity in many features shall still remain.

Aside from this similarity of the mutants to the parent phenotypically , the variant should also be similar in traits governed by several different gene complexes like quality characteristics, disease and pest resistance and biological characteristics as in several methods of protein identification.

11.6 ASEXUALLY PROPAGATED CROPS

Induced mutation is very useful to improve asexually propagated crops particularly for those that do not flower and those that take long period to flower.

Chimera formation however, becomes a problem because the nature of mutation which is a one-cell event. Thus when multi-cellular meristem is irradiated, the mutated cell has to compete with the un-mutated ones and this is called diplontic selection. Usually solid mutants are not expressed readily.

The problem of diplontic selection can be overcome by cutting back the top portion of the plant to force out the development of the side branches. When solid mutants are observed, they can be isolated through cuttings or tissue culturing to develop them into new plants.

Another approach is the use of adventitious bud techniques. This is based on the knowledge that the apex of adventitious buds originates from only one epidermal cell, thus solid mutants or homo-histant mutants can be recovered.

Chimera formation, however, is not a problem in ornamentals because any variation in color form are considered beneficial.

11.7 Yield Testing of Mutants

For seed crops, once the mutants are stabilized (M_2 to M_4), performance test should be conducted to find out the yield potential of the mutant(s). This is carried out in several locations and climatic conditions to find out the adaptability of the mutants. The entries should include the original variety/cultivar and a standard variety for comparison. A randomized complete block design with at least four replications will be sufficient. For testing two or more factors, split-plot design would be useful.

Part II

APPLIED MUTATION BREEDING

The categories now known as Basic and Applied Research did not exist prior to the 21st century. A number of researchers/scientists practice both basic and applied research. Basic and applied research while distinct in many ways, tend to merge in the process of scientific discovery. Today, basic research involves theoretical or experimental investigation to advance scientific knowledge, without immediate practical application as a direct objective, whereas the goal of applied science is essentially to create a situation that will eventually lead to a product or service being developed. But applied research uses knowledge gained through theoretical or experimental investigation to make things or create situations that will serve a practical purpose.

The scientific community comprises of researchers in both basic and applied research. Their research projects overlap and feed one another. It is difficult to make clear distinctions between basic and applied research just by looking at the results. Whether researchers call their work "basic" or "applied" is more a reflection of how they picture themselves and how much they aspire to interest with innovation system.

The difference between basic and applied science is narrowing rapidly, people who are working in applied research need to know the basics, because the demand for new, improved and better products that will feed the hungry world and increase the dwindling biodiversity which mutation breeding may help alleviate.

This practical section will follow the chronological discussions of agricultural commodities as presented by the previous Mutation Breeding Workshops/Seminars in an effort to combine all the methodologies observed to be useful for mutation breeding by the researchers of this aggrupation.

1. COMMODITY I: CEREALS

Cereals or grains are members of the grass family that are cultivated primarily for their starchy seeds or dried fruits (the grain) which is used for human food, feed for livestock and as a source of industrial raw materials. They are the most important food crops in the world. Rice, wheat, maize, barley, sorghum, oats, rye and millets are some of the most common cereals.

Cereals were among the first plants to be domesticated having been grown before the beginning of recorded history.

Rice, wheat and rye are grown primarily for consumption as human food while much of maize, barley, oats and sorghum and millet is mostly fed to livestock.

Rice is believed to have originated in the orient more than 5,000 years ago. It is the principal cereal grown for food in the tropics and warmer, humid parts of the temperate zone.

Wheat has been an important cultivated crop since prehistoric times. Many scientists believe it originated in Southwestern Asia.

Rye is botanically related to wheat and barley, all belonging to the tribe Hordeae. It was probably domesticated first in Turkestan having grown as a weed for many years in barley and wheat fields

Barley is a crop of ancient origin having been cultivated by Swiss lake dwellers in the Stone Age 11 and used for livestock feed and malt production and to some amount for human food.

Maize originated in the western hemisphere and was introduced into Europe and Asia by early explorers It is a highly domesticated crop that has been cultivated for 6,000 years or longer. It is the highest yielding grain crop.

Sorghum an important grain crop probably originated in tropical Africa. Its growth requirements and uses are similar to those for maize but is more heat and drought tolerant.

Oats are believed to have been cultivated first in Western Europe during the Iron and Bronze Ages. They are now grown mostly in temperate regions especially in corn belt of the US, Canada and in Northern Europe and in Australia.

1.1 RICE MUTATION BREEDING

INTRODUCTION

Rice (*Oryza sativa,* L.) is one of the two leading cereals in the world. It is feeding over half of the world's population. Rice is the staple food for the people of East, Southeast Asia, where 90% of the world's rice is grown and consumed.

Rice is grown in over 110 countries of the world, making 126 ha and 579 million tons of grain in 1990. Among the major producing countries, India leads in hectareage (29% of the world's total) while China follows in planted (23%) but leads in production (36%). Other Asian countries and India contribute about 56% of the production , while Latin America and African countries provide 3% and nearly 2% respectively. The United States contributes 1.35% to the world's crop. Thailand and the U.S.A. are leading exporters of rice and lately the Peoples Democratic Republic of Vietnam is exporting some of its harvest.

Rice provides more food energy yield per hectare than other cereals and the food protein yield per hectare is also high for rice because of its high mean yield. The superior quality of rice proteins provides a higher net utilization value of 73.8%.

In the rice-growing countries of the world, 83% of the rice land is irrigated; about 23% is rained-wet land fields followed by rain fed-dry land, 13%.

The genus Oryza belongs to the the tribe Oryzeae under the sub family Poo idea in the grass family gramine (Poaceae). The two cultivated species (cultigens) in the Genus are *O. sativa L.* (common rice) and *O. glaberrima* Steud (African rice).

The cultivated rice plants is characterized as an annual grass, with round, hollow, jointed culms, flat sessile leaf blades and a terminal panicle. The roots are fibrous, possessing rootlets and root hairs. It is a semi-aquatic plant, capable of both aerobic and anaerobic seed germination. It has aerenchyna tissues in the leaf sheaths and transport air to the submerged roots in the rhizosphere (by bacteria) and in the water (by blue green algae associated with azolla). The plant can supply a part of the nitrogen needed for plant growth from this non-symbiotic existence.

Some workers confused by certain terms in rice plants and its parts "paddy" refers to the grain or rough rice (in boiled form) and it also denotes the crop or harvested grains. "Paddies" refer to we land fields of rice. The inflorescence is a panicle. Rice seeds refers to the grain, the mature fruit (caryopsis) with its enveloping glumes (lemma, palea, awn, sterile lemmas) and the rachilla. The bulging sheath pulvinus is often mistakenly called the "node". In a germinating seedling, only the second leaf is a complete with sheath, blade, liqule and auricles. An adult plant height should include both culm length and panicle length. A grain is a filled spikelet.

Current advances in planting methods from direct seeding to transplanting and multiple cropping in a year from a single crop to two (main and off, kharif and rabbi) or three (aus, aman, and boro) have further enriched the genetic diversity in the rice of tropical and subtropical areas (Fig. II-1). Three ecogeographic races if rice are generally recognized, Indica, Sinica/Japonica and Javanica (Table II-1).

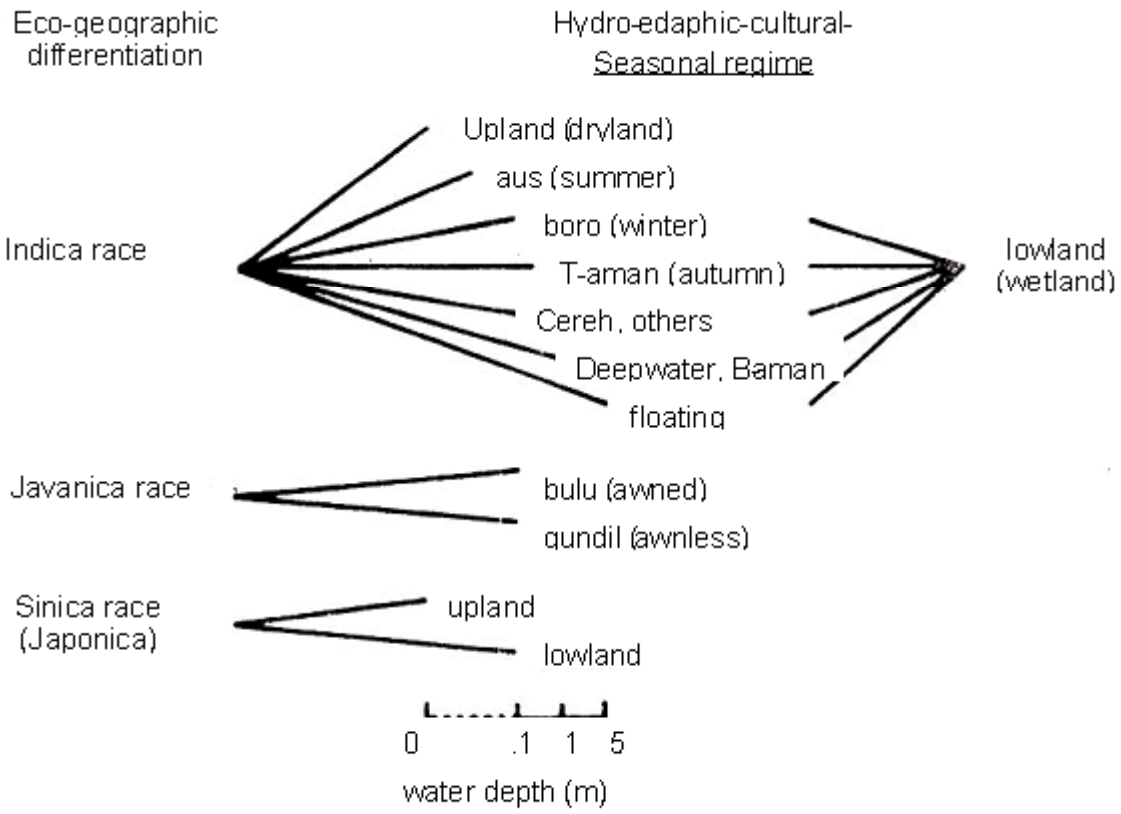


Fig.I-1 Complex array of varietal designations by topohydrologic, edaphic, cultural, and seasonal criteria (Chang, 1985a)

Table II-1
Ecogeographic races of *Oryza sativa*: comparison of their morphologic and physiologic characteristics

Indica	Sinica / or Japonica	Javanica
Broad to narrow, light green leaves	Narrow, dark green leaves	Broad, stiff, light green leaves
Long to short, slender, somewhat flat grains	Short, roundish grains	Long, broad, thick grains
Profuse tillering	Medium tillering	Low tillering
Tall to intermediate plant stature	Short to intermediate plant stature	Tall plant stature
Mostly awnless	Awnless to long-awned	Long-awned or awnless
Thin and short hairs on lemma and palea	Dense and long hairs on lemma and palea	Long hairs on lemma and palea
Easy shattering	Low shattering	Low shattering
Soft plant tissues	Hard plant tissues	Hard plant tissues
Varying sensitivity to photoperiod	None to low sensitivity to photoperiod	Low sensitivity to photoperiod
23-31% amylose	10-24% amylose	20-25% amylose
Variable gelatinization temperatures (low or intermediate)	Low gelatinization temperature	Low gelatinization temperature

*Semi dwarfs are mutants.

Objectives

Introduction to mutatioan for improved variety of rice with respect to any of the following traits:

I. Morphologic Traits Improvement (Fig. II-2)

- A. Pigmentation of plant parts
- B. Pubescence
- C. Dwarfs and semi-dwarfs
- D. Awning
- E. Panicle morphology
- F. Grain

II. Quantitative Traits Improvement (Fig. II-2)

A. Plant stature
B. Lodging resistance
C. Plant type
   1. Culm length
   2. Tiller number
   3. Culm angle
   4. Culm stiffness
   5. Leaf dimension
   6. Leaf angle
   7. Panicle characters

D. Grain characteristics
   1. Grain width
   2. Grain length
   3. Grain shape
   4. Grain thickness
   5. Heritability estimates and character asscociation
      a) Number of days from seeding to heading
      b) Spikelet length
      c) Plant height
      d) Panicle length
      e) Spikelet width
      f) Grain weight
      g) Number of grains per panicle
      h) Panicle weight
      i) Panicles per plant
      j) Tillers per plant
      k) Grain yield per plant

III. Growth Characters and Ecological Adaptation

`` A. Growth duration
   1. The basic vegetative phase
   2. The photoperiod-sensitive phase
   3. The optimum photoperiod where the shortest growth duration is obtained
B. Response to temperatures
C. Trait complexes contributing to wide-adaptability and high yielding ability
D. Growth habit
E. Grain shattering and dormancy
F. Tolerance to adverse environment
   1. Adaptation to deep water or floating ability
   2. Acidity
   3. Drought
   4. Temperature

IV. Hybridization

A. Grain yield and crop productivity
   1. Yield potential under most favorable condition
   2. Yield stability across seasoned locations
   3. Crop productivity on a daily field-use basis

B. Grain quality
   1. Physical appearance of grain (market quality)
      a) Size and shape
      b) Colour of brown rice or milled rice
      c) Test weight of rain
      d) Percentage of head rice (whole kernels) and brokens
      e) Transluscency
      f) Whiteness and chalkiness
      g) Cooking characteristics
      h) Purity and trueness to type or name
      i) Percentage of discolored or red kernels

   2. Cooking and eating quality
      a) Water absorption (kernel elongation and volume expansion during cooking
      b) Cohesiveness, texture and softness of cooked rice
      c) Hardness of cooked rice after cooking and upon cooling and standing
      d) Absence or presence of aroma
      e) Absence or presence of red rice
      f) Nutrition quality

C. Resistance to diseases and insects
D. Resistance and tolerance to hydrologic stress
E. Tolerance to extreme temperatures
F. Tolerance to adverse soil factors

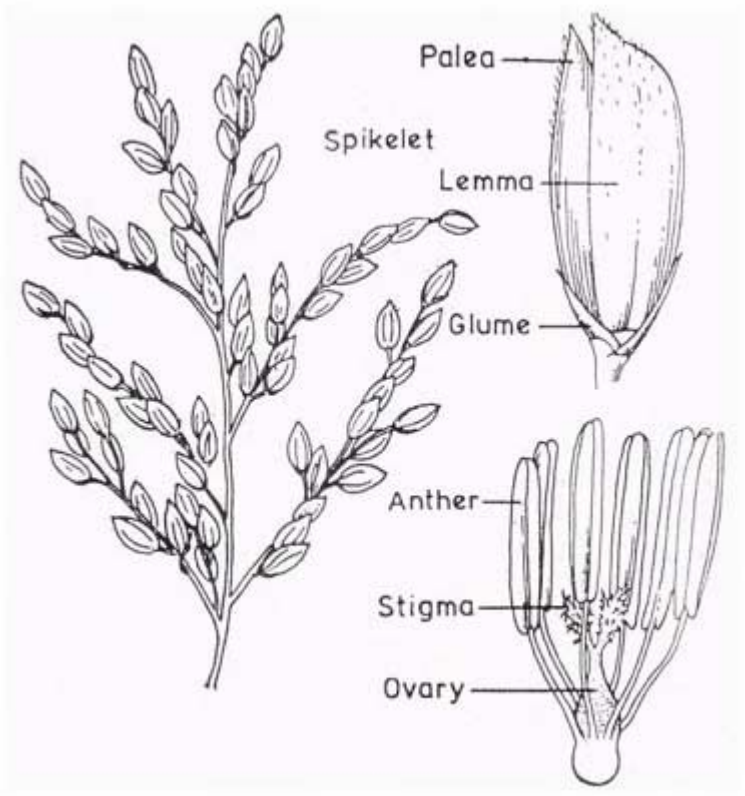


Fig. II-2 A part of the panicle of rice bearing single-flowered spikelets (left). A single spikelet (above, right) and a flower (below, right)

MATERIALS

Rice Seed (caryopsis), 5000 per envelope/dose
Manila (brown) coin envelopes at least 3" x 5" (7.5 x 12.8 cm)
Seed boxes with fine sand
Seed bed with garden soil-sand mixture (50 : 50)
Experimental paddies
Irradiation facility (Gamma cell 220, AECL), X-ray machine, etc.

METHODOLOGY

Select the base or parent variety in which mutations will be induced following criteria in Section I. Essential basic information and placed in envelopes at 5,000 seeds per dose and stabilized moisture between 13 – 14%. Expose each envelope to desired dose (20% higher and about 20% longer from that found in literatures or historical data of your laboratory for example 0.1 to 0.5 kGy. Set aside to 150 exposed seeds from each dose range for germination prior to replanting. For germination, seedling and survival tests, 50 seeds per treatment will be sown in basin filled fine sand. Sow the seeds closely within the rows and space the rows at 6 cm apart. Set up the experiment in a randomized complete block design with a number of germinations. Take percentage of germination after and seedling height measurement take survival data on the 21st day seeding.

For field planting, the seeds sown in seedbeds should be transplanted in the field on the 21st day after sowing. The seedlings (M_1) should be planted singly with spacings of 10 cm within the rows and 30 cm between rows. Record date of maturity. Harvest one panicle from the primary tiller of each plant in each dose at maturity, combine all panicles from each dose and plant again as M_2. Score chlorophyll mutation rates per 1000 seedlings/dose. Transfer M_2 seedlings singly in the field 20 x 25 cm apart between hills and rows. Score for normal morphologic mutations for preliminary yield trial seed of M_2, i.e.., M_3 should be sown in seedbed and transplanted 21 days after in a well-prepared field. Prior to final harrowing, 80-20-20 Kg, NPK per ha should be applied and incorporated during the final land preparation. Three to four seedlings should be planted per hill with spacing of 20 x 20 cm between hills and rows. At panicle initiation, 20 Kg N per ha must be applied. Keep the experimental field cleared by constant weeding and apply insecticide to control insect infestation. Design the experiment in a randomized complete block design with replications.

I. Early radiation effects should be observed at M_1
   1. Percentage germination
   2. Survival rate
   3. Growth (height) 7 days after sowing
   4. Growth (height) 14 days after sowing

II. Observation on the frequency of chlorophyll and morphological mutations at M_2

   1. Chlorophyll mutations per 1000 seedlings
      a. Striata
      b. Virescemt
      c. Albina
      d. Others

   2. Morphological mutation frequency (see list objective)
      a. Morphology
      b. Quantitative traits
         1) Stature
         2) Lodging resistance
         3) Plant type
         4) Grain characteristics

III. Yield performance of the mutants based on agronomic traits M_3
1. Days to heading
2. Nature plant height
3. Productive tillers
4. Panicle length
5. Number of grains per panicle
6. Percentage sterility
7. Weight of 100 grains
8. Yield (T/ha)

HANDLING OF M_1 GENERATION

a. TREATMENT CLASS

Always prepare small amount of control (zero – dose). This assures you a good handling during treatment and planting. If the growth is inferior to the usual operation, some mishandling might be there. If it is routine mutagenesis work, numbers of plant may be some 100 or less. It often helps you to preserve the original variety.

As a general rule, the smaller the treatment dose, the better. Although it requires larger population size, the unfavorable accompanied mutation can be suppressed to low level. Growth reduction by 20% (height, fresh weight, etc) or 80% growth, would be a recommended level. To be sure of the appropriate dose, some sample should be treated to find the 50% growth reduction dose, making at least 4 classes, e.g. control, 10%, 20%, and 50% or more reduction, the size of the population being e.g. 1, 10, 20 and 1. (Fig. II-3)

b. HARVESTING SCHEME

This depends on the crop species, or how the seed are produced. Numbers of seeds in one unit (panicle, spike, ear, capsule, fruit, etc) and M_2 population plan must be considered.

Planting of M_2 plants

M_1 plant basis	Pooled within M_1 plant. Many chimeric sector gives high Frequency of mutants
M_1 spike basis	Pooled within spike etc, Generally easiest to handle
M_2 seed basis	Pooled within M_1 population. Similar mutants might be from the same mutation

You may use the last method by dividing the M_1 population into small subgroups of about 300 plants or so, so that similar mutants in different subgroup can be assured to be derived from independent mutation.

Application

Size of 1 unit (beware of chimera within the unit)

Rice, millet	100 seeds or more
Barley, wheat	20-50 seeds
Beans, peas	5-20 seeds/capsule, 1-3 capsule per bunch
Soybean	1-3 seeds per capsule, 2-4 capsule per node.

Harvest scheme used for rice (E. Amano) (Fig. II-4)

Plant M_1 as ordinary planting pattern
Harvest 3 rounds, 1 panicle at a time from each M_1 plant
1st harvest is for endosperm character screening
2nd harvest is planted to the field as M_2 generation (panicle basis)
3rd harvest is stored in cold storage for future use

In case of japonica race which is non-shattering, panicles can be tied together until use, but for other shattering crops, paper bags or other storage containers will be necessary in large numbers.

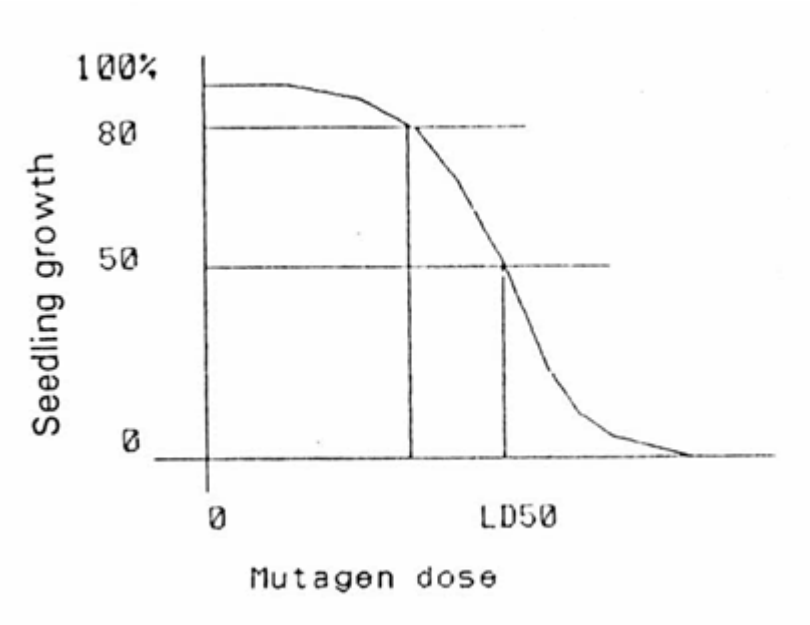


Fig. II-3 Dose-survival responce
LD50 is good to define the sensitivity, but 80% survival may be recommended for better surbival and less accompanied unfavorable mutation.

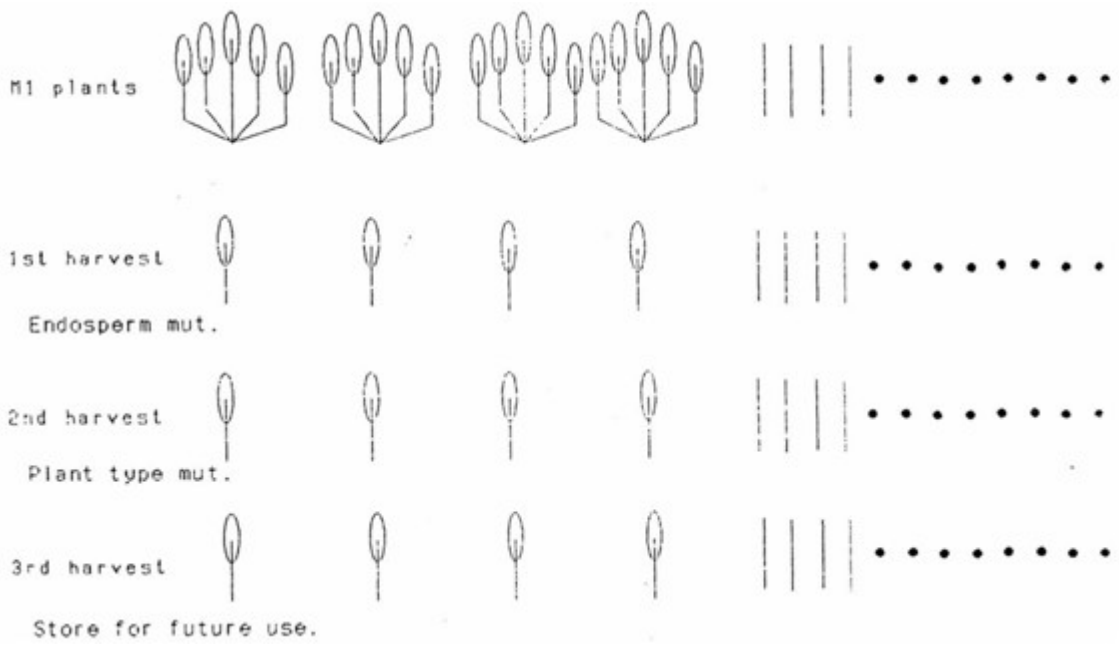
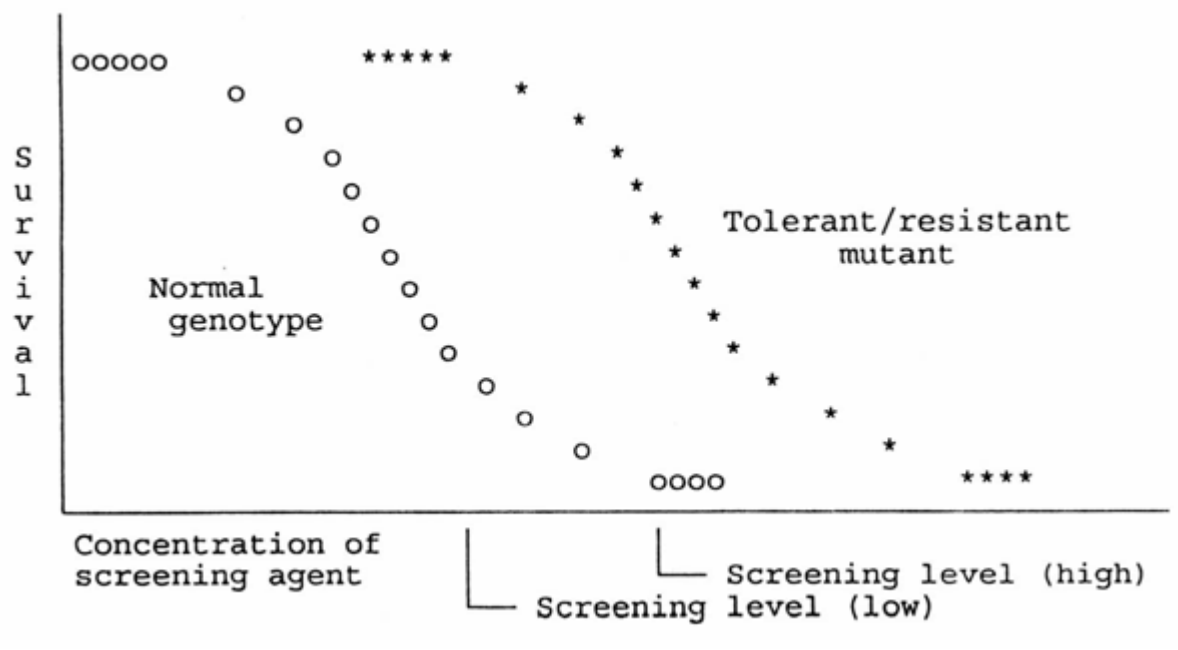


Fig. II-4 Example of harvest method (rice)

c. SCREENING/SELECTION METHODS

CONCEPT OF RESISTANT/TOLERANT SCREENING (SURVIVAL SCREENING)



Factors to be considered :

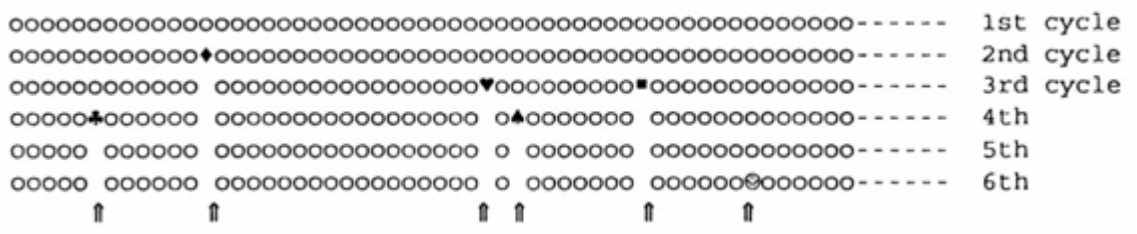
- uniformity of the screening agent (including temperature, etc.)
- Resasonable selection level
- Environmental condition
  Light, water, humidit, air circulation
  Field, greenhouse or laboratory

Isolation of a few generations before the mutagenesis work is recommended to ensure the purity of the material.

SCREENING BY MEASUREMENT

Protein quality and other biochemical component must be measured one by one. The method used in selection of rice protein mutant by electrophoresis assay from 1600 M_1 panicles was as follows :

- One panicle (one from each M_1 plant) was considered as the unit of mutation.
- One grain from each panicle was assayed in each assay cycle.
- If variant in electrophoreogramme was found, the panicle was separated for further analysis
- Grain analyses continues for 8 cycles



(Prepared by E. Amano)

FURTHER EXAMPLES OF MUTANT EVALUATION

GRAIN QUALITY EVALUATION

The development of rice varieties provide more choices to farmers, traders, millers, processors as well as household consumers who in turn become more selective. The grain quality plays a key role in the assessment of selections according to standards that are beneficial to the various sectors concerned.

The project specifically provides information on grain quality characteristics of rice selections. These characteristics include : 1) milling potential (% brown rice, % total milled rice, and % head rice), 2) physical attributes (% chalkiness, % immature grains, grain length and grain shape); 3) physico-chemical properties (%amylose, gel consistency, gelatinization temperature and % crude protein); 4) cooking parameters (optimum cooking water, % height increase and cooking time), and 5) sensory qualities (% acceptability, preference score and descriptive characteristics as aroma, flavor, tenderness, color, gloss, cohesiveness, translucency and brittleness of grains).

METHODOLOGY

Rice samples for grain quality tests come from the breeders usually starting from the preliminary yield trial. Standard yield check varieties for each group and a sensory quality check are grown along with the entries. Rice samples are aged for three months before actual analysis. Breeding materials in the earlier generation are usually screened for kernel quality along with the agronomic and field reactions to insect pests and diseases.

The flow chart of grain quality evaluation is shown in Fig. II-5.

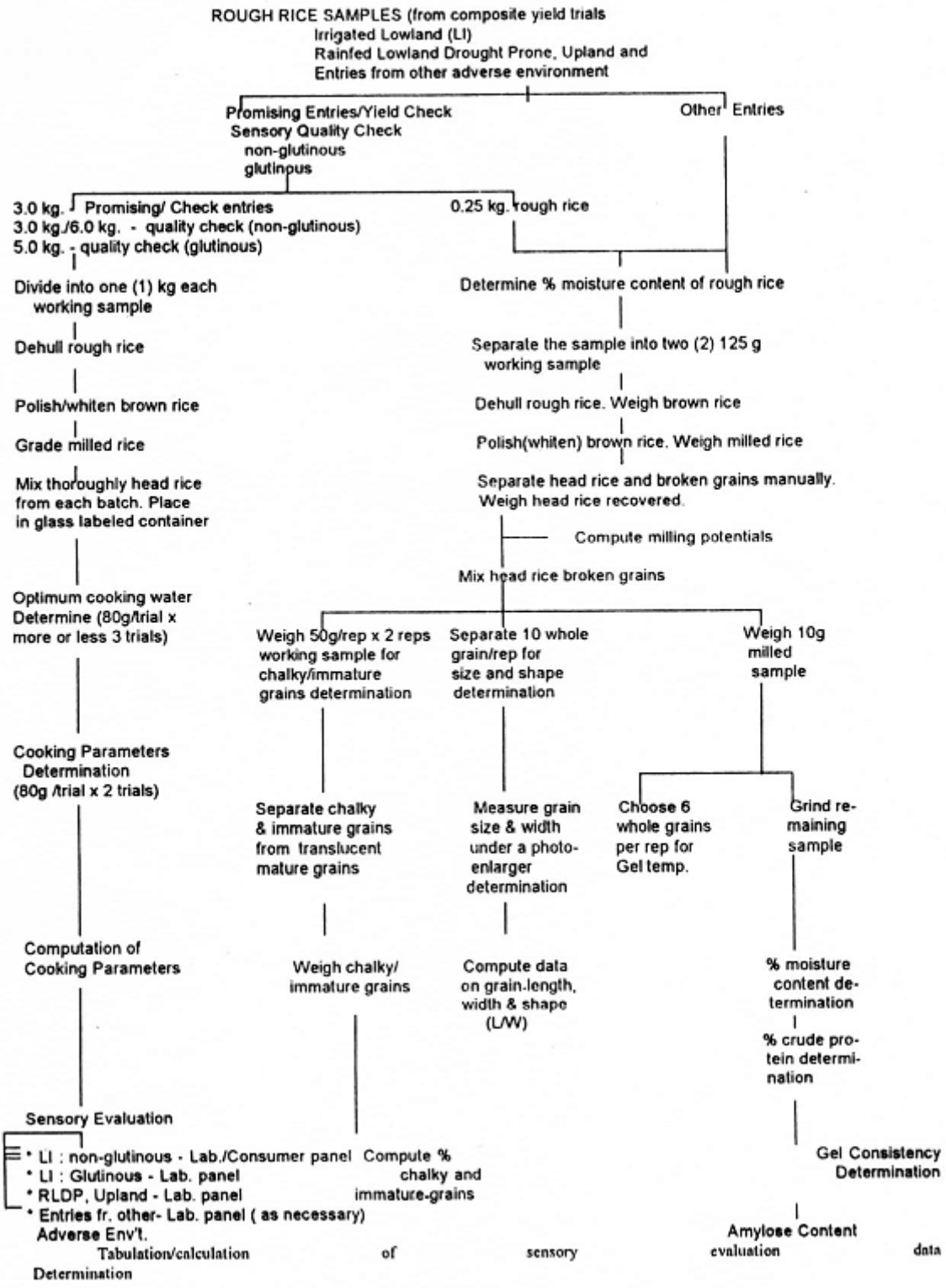


Fig. II-5 Flowchart of Grain Quality Evaluation of Rice Entries

a. MILLING POTENTIALS

Milling yield of rough rice is the estimate of the quantity of total milled rice consisting of head rice and broken grains that can be produced from a unit of rough rice. It is generally expressed in percentage.

The percentages obtained are classified based on a scheme developed from research experiences and readings. For each of the parameter, a recommended value/classification is used as reference for varietal recommendation as shown in Table 2.

Table II-2. Classification and recommended value for parameters of milling potentials

Milling Potentials	Classification		Recommended Value
% Brown Rice	Good (G)	80.0% and above	75.0% and above
	Fair (F)	75.0 – 79.9%	(Fair to Good)
	Poor (P)	below 75.0%	
% Total Milled Rice	Premium (Pr)	70.1% and above	65.1% and above
	Grade 1 (G1)	65.1% – 70.0%	(Grade 1 to Premium)
	Grade 2 (G2)	60.1% – 65.0%	
	Grade 3 (G3)	55.1 – 60.0%	
% Head Rice	Premium (Pr)	57.0% and above	48.0% and above
	Grade 1 (G1)	48.0% – 56.9%	(Grade 1 to Premium)
	Grade 2 (G2)	39.0% – 47.90%	
	Grade 3 (G3)	30.0% - 38.9%	

b. PHYSICAL ATTRIBUTES

The physical attributes consists of four (4) parameters namely: % chalky grains, % immature grains, grain length and grain shape. Chalky grains are whole or broken grains, one half or more of which is white like the color of a chalk and is brittle. Immature grains are light green and chalky with soft texture. Grain length, on the other hand, is the length in millimeters of the rice grain, while grain shape is the ratio of length and width of the grains.

Grain Length (in mm)		Grain Shape (length/width in mm)	
Extra Long (EL)	7.5 and above	Slender (S)	more than 3.0
Long (L)	6.6 – 7.4	Intermediate (I)	2.0 – 3.0
Medium (M)	5.5 – 6.5	Bold (B)	less than 2.0
Short (S)	5.4 and below		

Results of the consumer preference studies for more than 20 years revealed that Filipinos prefer rice grains which are long and slender with grain size of 6.6 mm to 7.4 mm and grain shape of more than 3.0.

Table II-3. Classification and recommended value for % chalky and immature grains in rice

Classification			Recommended Value
% Chalky Grains	Premium (Pr)	< 2.0 %	less than 5.0 %
	Grade 1 (G1)	2.0% - 5.0%	(Grade 1 to Premium)
	Grade 2 (G2)	5.1% – 10.0%	
	Grade 3 (G2)	10.1% – 15.0%	
% Immature Grains	Premium (Pr)	< 2.0 %	less than 2.0 %
	Grade 1 (G1)	2.0% - 5.0%	(Premium)
	Grade 2 (G2)	5.1% – 10.0%	
	Grade 3 (G3)	10.1% – 15.0%	

c. PHYSICO-CHEMICAL CHARACTERISTICS

The physico-chemical characteristics of the rice grains are important indicators of grain quality. They are referred to as indices of cooking qualities and cooked rice texture. These characteristics are gelatinization temperature, gel consistency, amylase content, and crude protein.

1. Gelatinazation Temperature. Gelatinization temperature is estimated by the extent of alkali spreading of raw milled rice soaked in 1.70% (anhydrous) potassium chloride for 23 hours at 30 ^{o} C or at room temperature. Thus, rices with low gelatinization temperature disintegrate completely, whereas rices with high gelatinization temperature remain largely unaffected in the alkali solution.
2. Crude Protein Determination. Rice grain contains 8 – 10% protein. It is an important factor from the viewpoint of nutrition and taste. There is a tendency of decreased palatability as the content of protein increase (Ishima et al, 1974). Very common among Philippine varieties register 6- 7 % protein.
3. Amylose determination. Amylose and amylopectin are major ingredients of starch of non-glutinous and glutinous rice, respectively. It is also closely related to the palatability of rice. Varieties with high amylose content are less sticky and fluffy while low amylose rices are more sticky.

Classification of rice according to amylase content is listed below

Waxy/glutinous	(w)	- 1.0% - 2.0%
Very low	(vl)	- 2.1% - 10.0%
Low	(l)	-10.1% - 20.0%
Intermediate	(i)	-2 0.1% - 25.0%
High	(h)	more than 25.0%

For Filipinos, intermediate amylose content is most preferred.

4. Gel Consistency Determination.— Gel consistency is a measure of the flow characteristics of milled rice gel. The test differentiates in terms of tenderness of cooked rice. Within high amylose group, varieties with soft/medium gel consistency are more tender when cooked than rices with hard gel consistency. The test separates rice into the following classification :

Rating	Length in mm
Hard	25 - 40
Medium	41 – 60
Soft	61 – 100

d. COOKING PARAMETERS AND SENSORY EVALUATION OF MILLED RICE

The milling and cooking procedures affect the quality of the milled raw and cooked rice grains, hence a standardized method to evaluate the cooking parameters and sensory attributes is very necessary. Components for cooking parameters include optimum cooking water, height increase and exact cooking time.

Sensory evaluation is used to interpret sensations perceived by the human senses of sight, smell, touch and hearing. It is conducted using either or both trained laboratory and consumer panels.

Laboratory Panel	Consumer Panel
1. Measures sensory differences. Describes or characterizes a food product in terms of cooked rice attributes as aroma, color, flavor tenderness, gloss, cohesiveness and texture as well as raw rice attributes like aroma, color, gloss, translucency of grains, wholeness of grains and brittleness of grains.	1. Measures preferences and acceptability (likes or dislikes) preference where choice is involved. General characteristics are considered.
2. The number of panel members is small. Consist of five (5) to ten (10) panelists	2. The number of panel members is large Usually 30 to several hundred of persons as the case warrants.
3. Trained. The members are selected from a large group for their acuity and consistency in recognizing differences.	Untrained. The ability of its members to discriminate according to degree for specific attributes is not considered as long as they are representative of the consuming public and are willing to participate.

Based on the above considerations, preference is determined by ranking and is expressed in scores of ranked data following the procedure of Lamond (1985). Acceptability is indicated by a "yes" or a "no" response.

1.2 MUTATION BREEDING IN WHEAT

INTRODUCTION

Wheat (*Triticum aestivum*) is one of the two leading cereals in the world. The total wheat area harvested worldwide in 1992 was 220 million ha. Representing 31.4% of the global area planted to cereals. Wheat area harvested in developing countries has 100 million ha or 23.5% of the cereal hectareage for developing countries. Increases both in yield and area in the last two decades in the developing countries are due to the spread of high yielding semi dwarf wheat varieties, the expansion of irrigation and the increase in use of chemical fertilizers. The Third World's share of global bread wheat production has increased from 27 to 41% in the past 40 years. In the developing countries, the research on yield is a high priority where mutation breeding can perhaps contribute to augment existing programmes (Fig. II-6).

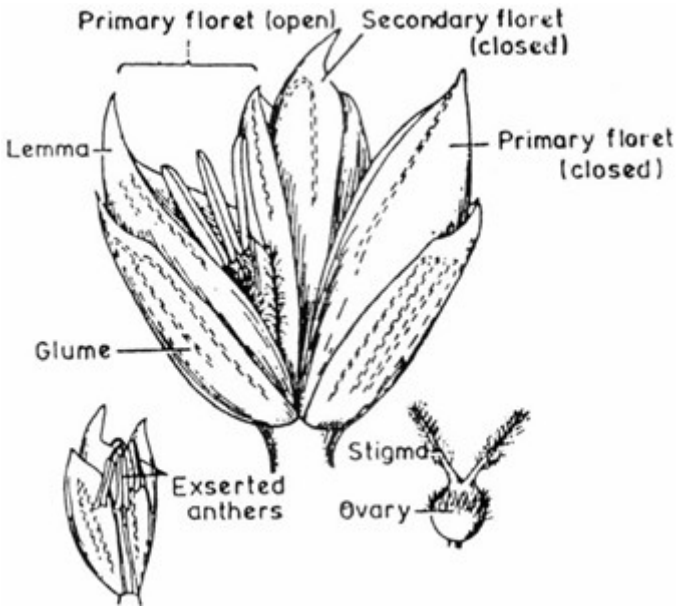


Fig. II-6 Spikelet of wheat (center) in which the primary floret on the left is open showing the three anthers and a part of the feathery stigma. Another primary floret and the secondary floret are closed. On the left is a floret showing anthers excerted after blooming and on the right is the pistil with two feathery stigmas.

OBJECTIVES FOR MUTATION BREEDING

1. Breeding for high genetic yield potential, stability, and wide adaptability
2. Breeding for pests and diseases resistance/tolerance
3. Breeding for drought tolerance
4. Breeding for mineral stress conditions
5. Breeding for salt tolerance and other abiotic stresses

MATERIALS AND METHODOLOGY

MATERIALS

1. Wheat seeds 5,000/dose
2. Manila (brown) coin envelopes to contain seeds for irradiation
3. Seed boxes
4. Seedbed
5. Experimental field
6. Irradiation facility
    a. Co-60 or Cs-137, etc.
    b. X-ray machines, etc.

METHODOLOGIES

1. Determine radiosensitivity of seeds of selected variety to establish lethal dose
2. Starting from lethal dose, expose seeds at incremental decrease of radiation doses
3. Germinate in seedbox for response studies
4. Plant in seedbed for germination prior to field transplant
5. Transfer to experimental field

2. COMMODITY II: LEGUMES

DATA GATHERING AND SOURCES OF DATA

M_1 - Materials are space planted. Outstanding plants are identified and selected on the basis similar to rice breeding (i.e. percentage germination, survival rate, growth after sowing,etc). Transfer seedbed planted to experimental plots. Selection are advanced to M_2.

M_2 - Two to three thousand plants are grown with space in M_2 in well watered, high fertility conditions. Individual plants are characterized for disease resistance and agronomic suitability (i.e. tillering, lodging, maturity, etc).

M_3 - Progeny of selected M_2 are grown solid in 2 m three-row plots. Each plant is treated as a separate population. Selection is done by plot first and individual heads in the selected plot are bulked. The environment of the M_3 field depends on the conditions for which materials are being selected. Irrigated or rainfed environments, acid soils, hot climate, etc.

M_4 - Bulk of M_4 is planted solid and selection criteria and choice of environment are the same as M_3.

M_5 - Approximately 100 plants/plot selected in M_4 are space planted in a favorable, high yielding environment. Individual plants are selected on the basis of disease resistance, acceptance, agronomic characters and spike fertility.

M_6 - Each plant selected in M_5 is grown individually as M_6 in plots of three rows, 2 m long. Agronomically outstanding lines acceptable in resistance (diseases, pests, environmental) are bulked in advance to yield evaluations on the basis of choice in rainfed, acid soil and hot humid climate environments according to breeding objectives. The lines developed in purely irrigated condition of high fertility are also checked in rainfed condition of low fertility and vice-versa.

BREEDING MUNGBEAN

The mungbean breeding program of the PNRI dates back to the 60s when Dr. I.S. Santos, the former Head of the Agricultural Research Section, isolated a multifoliata mutant after seed treatment of the small seeded, shiny yellow mungbean variety with EMS (ethyl methyl sulfonate), at 0.04 M. At first, the mutant appeared to have no economic value being small seeded, had poor seedling vigor, very leafy and late maturing. Being leafy however, the mutant appeared to be useful only as green manure crop to improve soil fertility. While studying its later progenies, it was observed that this mutant possessed some degree of male sterility or reduced cleistogamy - allowing natural outcrossing with other varieties. To mungbean breeders, the trait appeared very useful since conventional breeding by emasculation was difficult. This unique trait allows transfer of the mutated gene into different genetic backgrounds, thus allowing introduction of desirable genes into other varieties. The discovery urged plant breeders to work further for its improvement to produce the variety with the needed genotypes.

Thus, starting in 1972, a cross breeding program was initiated to improve the mutant by natural outcrossing using mungbean varieties recommended by the Philippine Seedboard. Varieties developed by the Bureau of Plant Industry and the University of the Philippines at Los Banos were used as parents of the crosses made. In 1975, after several plantings, observation and selection, a large-seeded variety was isolated. It was early-maturing, non-lodging, less leafy and had shiny yellow seedcoat color. Further selection resulted in the development of the many mungbean varieties developed at the Institute. A schematic diagram of the breeding procedure is illustrated below.

Original Parent
(small-seeded, native variety) ----------→ Original Multifoliata mutant
EMS (0.04M)

Crosses Made :

Orig. Mutant X CES 14 Selection
CES 28 -------→ for desirable plant types
CES 55
(U.P. Los Banos Varieties)

Orig. Mutant X MD 15-2 -------→ Selection for desirable plant types
MGS-10 A
(Bureau of Plant Industry varieties)

F_7 of the cross : MG50-10A X Orig. Mutant -------→ Large-seeded Multifoliata was isolated

Further selection and purification resulted in the development of the following varieties :

PAEC 1 - Medium-size seed : shiny yellow seedcoat; matures in 70 to 75 days; yields about 10% less than the high yielding Seedboard recommended green-seeded CES 55; should not be grown in May to July, because it grows too tall, and sets very few pods; recommended for planting in November to January when it grows taller than most mungbean varieties and therefore competes well against weeds.

PAEC 2 - Multifoliata leaf; some seeds have shriveled or wrinkled seedcoat; seeds as small as those of the yellow native mungbean; matures later than PAEC 1 by about five days, grows about 10 cm shorter and yields about 5 to 10% less; recommended only for green-manuring; might have superior eating quality and/or nutritional value because of the wrinkled seedcoat.

PAEC 3 - Large-seeded; seedcoat very shiny yellow; matures in 60 to 65 days; in the dry season planting for which it is recommended, grows to a desirable height of 40 to 60 cm and yields as much as CES 55

PAEC Mungbean No. 5 - Seeds as small as those of native yellow mungbean; yelds as much as CES 55 in the dry season; Recommended to replace the still-popular small-seeded native yellow mungbean which has the undesirable characteristic of shattering pods; grows to a very desirable height of 40 to 60 cm.

PAEC Mungbean No. 7 - Very large-seeded; matures in 60 to 65 days; grows to a very desirable height of 40 to 60 cm and yields a little less than CES 55. Recommended for growing in the dry season for sotanghon production.

PAEC Mungbean No. 9 - Dull yellow seedcoat; medium-size seed; matures in 60 to 65 days; with wider and longer pods than PAEC Mungbean No. 7; grows to a desirable height of 40 to 60 cm and yields at least as much as CES 55. Recommended for use by farmers who would want to produce their own pure seeds

PAEC Mungbean No. 10 - Multfoliata leaf; otherwise it has the same characteristics as PAEC Mungbean No. 9. Unlike PAEC Mungbean No. 2 (multifoliata), has vigorous, fast growing seedlings and does not have the yield depression associated with the multifoliata leaf. Recommended for growing in shady areas and for green-manuring. Even easier to maintain pure than PAEC Mungbean No. 9.

3. COMMODITY III: ASEXUALLY PROPAGATED ORNAMENTALS

INTRODUCTION

Ornamentals have become an important commodity, a promising dollar earning crop in the country. It has a big market potential both here and abroad. Development of improved varieties of these ornamentals through mutation breeding will enhance export capacity, which will greatly help in the country's economic recovery.

Furthermore, this will serve as encouragement for enthusiasts to grow more of these ornamental crops that would create livelihood opportunities. This will help also in realizing the goals of becoming an export winner and sunshine industry.

OBJECTIVES

General : To develop improved varieties of ornamental crops

Specific:
1. To develop a wide array of cutflower (orchids and chrysanthemum) with improved characteristics (color, texture, substance) earliness to flower (Fig. II-7)

2. To induce variation in form or color of the leaves, size and growth habit in Foliage and plants that will cater to changing consumer demands. (Fig. II-8)

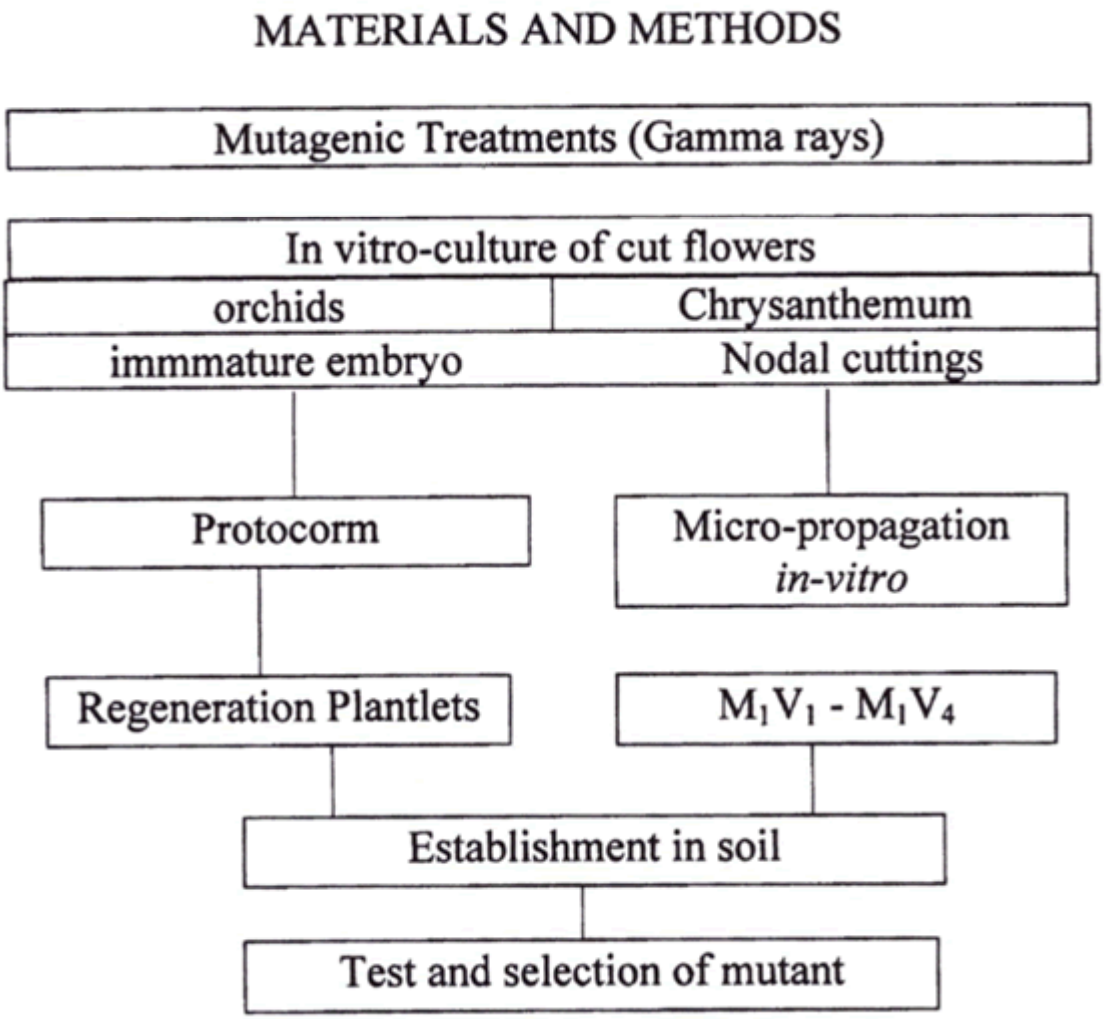


Fig. II-7 Schematic diagram for mutation breeding in cut flower ornamentals

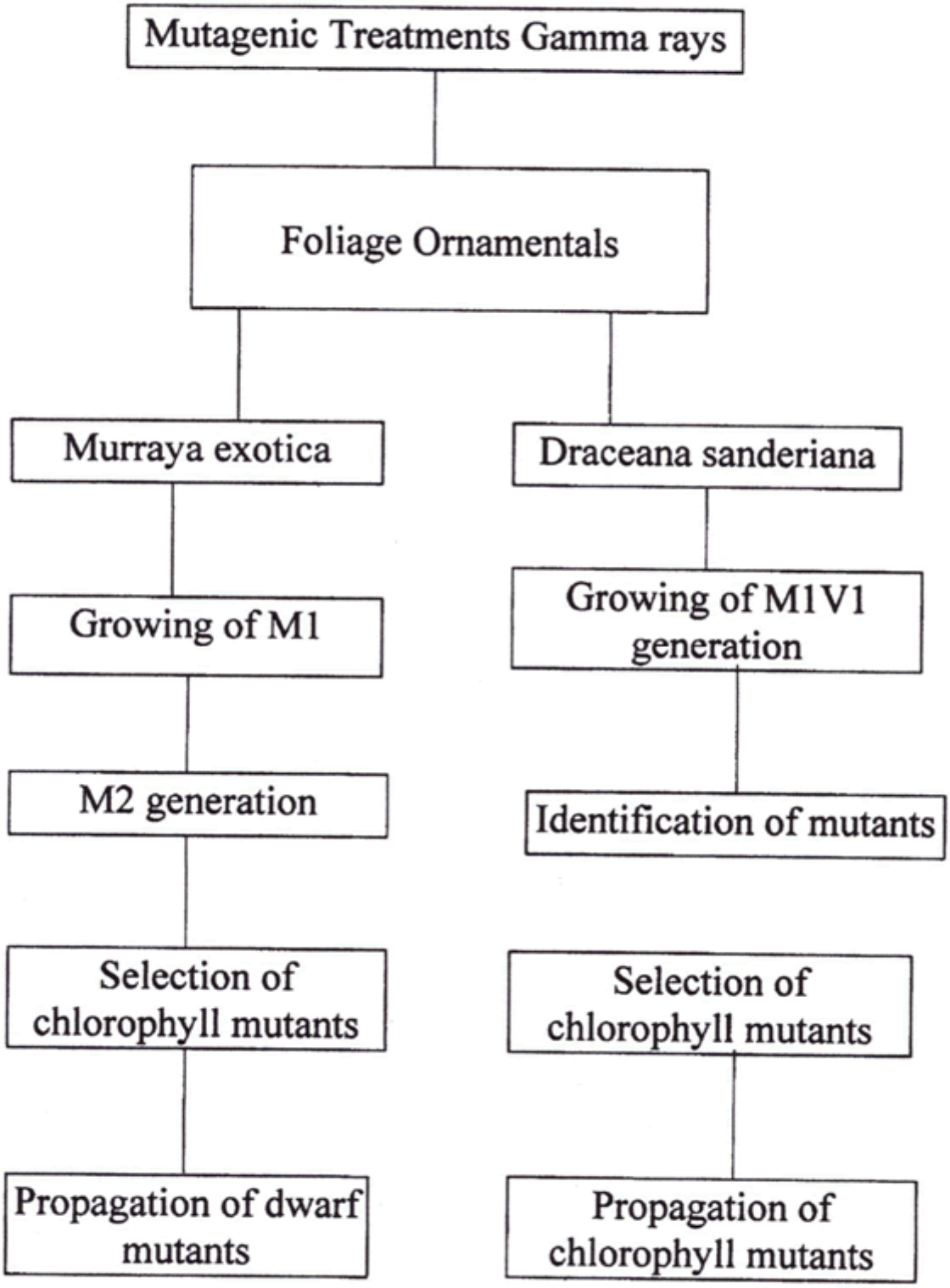


Fig. II-8 Schematic diagram of mutation breeding of foliage ornamentals

3.1 CUTFLOWER ORNAMENTALS

Studies on the tissue culture of orchids and chrysanthemum are presently being done to induce variation and to provide a rapid means of propagation.

Murashige and Skoog's (MS) medium is used for the *in-vitro* micropropagation of chrysanthemum. Irradiated and non-irradiated stem explants were planted in MS medium for the regeneration of plantlets. Sub-culturing of the plantlets were done until roots are well-developed. Whorling and changes in leaf color were observed at 10 Gy and doubling of the leaf growth at the node in 20 Gy of the 3rd vegetative generation. Explants (shoot tips and nodal sections) were obtained from this generation and planted in MS medium as M_1V_4 generation. At present, there 783 seedlings micropropagated. Morphological changes observed in the fourth generation were multiple branching per node in the 10 Gy dose as well as in the 20 Gy dose.

For orchid tissue culture (Fig. II-9a-d), the Knudson C (KC) medium with organic additives is used for embryo culture. Irradiated immature embryo were inoculated in KC medium and allowed to develop into callus and protocorms. There was proliferation of growth of callus and protocorm tissues with repeated subculture in *Dendrobium pattaya* Beauty. Protocorms are sub-cultured for plantlet regeneration.

In *Vanda sanderiana*, reflasking of the plantlets was done until roots are well-developed and ready for compotting. Seedlings were compotted in orchid pots containing osmunda fiber and charcoal and grown under greenhouse conditions. A decrease in seedling height, number of roots and rootlength was observed with increasing dose of gamma radiation of 10 and 20 Gy.

MURRAYA EXOTICA (Local name: Kamuning)

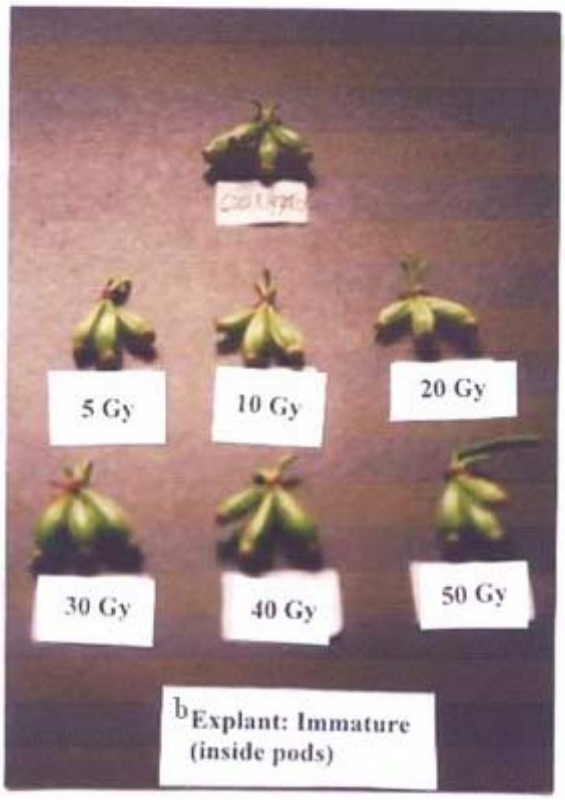
Seeds of *Murraya exotica* (local name, Kamuning) was obtained from sexually precocious plant selection of Dr. Ibarra S. Santos were exposed to 10, 20, and 30 Gy dose of gamma rays. These were sown in seedbed and grown under greenhouse conditions. Seeds were germinated 45 days from day of sowing and bore flowers from 30 to 60 days. Seeds that developed from M_1 generation were harvested and grown as M_2 generations. These were isolated and grown as M_3 generation. Selection and purification of mutants were done in the M_4 to M_7 generations. In the M_7 generation, a dwarf mutant (1 to 2 feet) was obtained from a 30 Gy dose of gamma rays. This mutant is characterized with branching and spreading habit of growth in multi-flowered chymes that are prolific. Unirradiated Kamuning usually grows to a height of 7 feet or 210 cm.

The dwarf *Murraya* mutant was approved for registration by the Technical Working Group in Ornamentals.(Fig. II-10)

DRACAENA SANDERIANA (Local name: Corn plant)

Stem cuttings of *Dracaena sanderiana* (local name, corn plant) were exposed to 10 and 20 Gy of gamma rays and planted in seedbeds and grown under greenhouse conditions. M_1V_1 generation cuttings were obtained in the M_1V_1 plants and planted as M_1V_2 generations. Chlorophyll mutants were induced in the M_1V_2 generation. The mutants were selected and grown in the M_1V_3 and succeeding generations. In the M_1V_7 generation, a *Dracaena sanderiana* mutant was induced. (Fig. II-11a.b) It is a stiff and robust plant with modified shape and stripes of the leaves. The center is silver-green with white stripes and broad margins in deep green.


a. Orchid: *Vanda sanderiana*
(waling-waling)


5 Gy
10 Gy
20 Gy
30 Gy
40 Gy
50 Gy
b Explant: Immature
(inside pods)

c. **Vigorous plantlets at 10 Gy in *Vanda sanderiana***

d. **Regeneration**
Plantlets for compotting

Fig. II-10 ***Dwarf Murraya*** **at 20 Gy**

Fig. 11a **Chlorophyll mutations in *Draceana sanderiana*: Induction of greenish yello to yellowish green color of the leaf with green dots (Muricata)at 20 Gy.**

Fig. 11b **Chlorophyll mutation in *Draceana sanderiana*: Induction of white color of the leaf changes from yellow green to dark at 20 Gy.**

3.2 ORNAMENTAL PINEAPPLE

INTRODUCTION

Pineapple (*Ananas comosus* (L.) Merr) is one of the most important crop of the Philippines. The pineapple industry provide employment and income to thousands of Filippinos.

The pineapple Queen variety is the most desirable for fresh consuption because of its sweetness of fiber content and crispiness of the flesh. However one objection to this variety is the presence of troublesome hard spines which interfers with cultivation, weeding and harvesting.

Mutation breeding through the use of ionizing radiation (gamma rays) coupled with *in-vitro* culture techniques was undetaken for the developement of improved pineapple Queen variety with the following objectives:

(1) To eliminate or reduce spines/thorns along the margin of the leaves. (Fig. II-12, 13a,b)
(2) Progation of the chlorophyll mutation which can be the bases of ornamental types
(3) To establish protocl for the tissue culture techniques for the Queen variety pineapple.
(4) To make use of the *in vitro* culture techniques as a tool for induced mutation breeding.

Induction of mutations, using ionizing radiations in vegetively propagated crops, one of the problems encountered is diplontic selection and chimera formation which lower the efficiency of mutation breeding. This problem can be avoided by developing a single-cell approach which can be made possible by the development of cloning techniques through tissue culture and the use of axillary buds. Buds consist of few cells provide larger mutated sectors in the growing shoot. Hence, the use of the *in-vitro* approach (through irradiation of axillary buds) and the *in-vitro* technology (through tissue culture techniques) were the methods used to attain the induction above-mentioned objectives.

MATERIALS AND METHOD

a. MATERIALS

Pineapple (*Ananas comosus* (L.) Merr.) variety Queen obtained from Imelda, Camarines Sur, Bicol Region was used as tthe test material for mutation studies. Crowns of uniform size were selected, and the bud at the base of each leaf was separated carefully by a scalpel, taking into consideration not to separate the bud from leaf.

b. *IN-VITRO* CULTURE APPROACH

Establishment of the protocom for the tissue culture of pineaple Queen variety

Protocol:

1. Crowns of pineapple variety Queen were used as sources of explants.
2. Crowns were washed in tap water with detergent to get rid of soil particles.
3. Leaves were removed from the crown and surface-sterilized with 70% ethanol for 1 minute and in 5.25% sodium hypochlorite for 20 minutes and rinsed several times with sterile distilled water.
4. Crown sections without buds of about 0.5 cm^3 were inoculated in soil Murrashige and Skoog (MS) medium with 10% coconut water (cw), sucrose and varying levels of benzyl adenine (BA) in combination with naphthalene acetic acic (NAA), autoclaved prior to inoculation at 15 psi for 20 minutes at 120° C
5. Callus from the crown section were induced with the best treatment combination of BA and NAA that would enhance plantlet morphogenesis
6. Series of sub-culturing in fresh medium were done until plantlets were ready to be transferred to the soil.

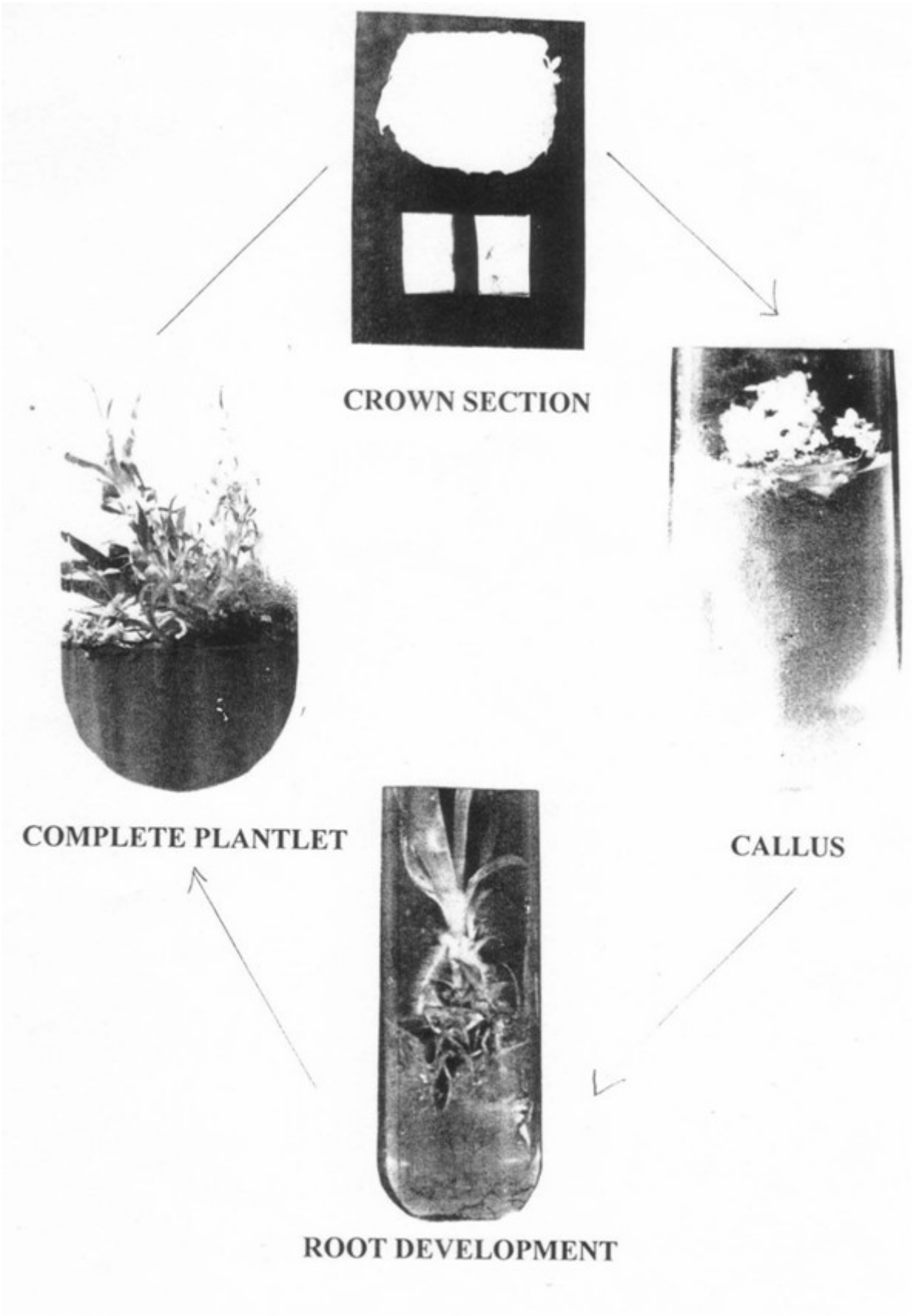
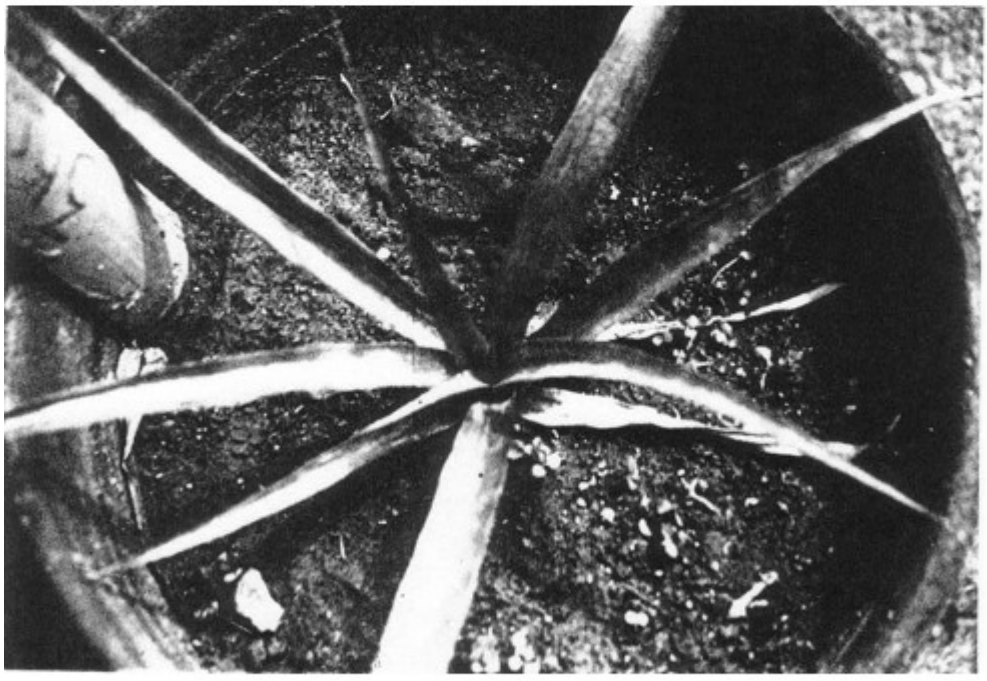


Fig. II-12 Stages of Development

(a)



(b)

Fig. II-13 Mutants induced in pineapple
(a) chlorophyll mutant
(b) plant with reduced spines

c. IRRADIATION TREATMENT

Eighteen lots were divided into 6 groups and irradaited with dose levels of 10, 20, 30, 40, and 50 rad of gamma rays from a Cobalt-60 facility of the Philippine Nuclear Research Institute, Diliman, Quezon City. The remaining non-irradiated axillary buds were used as control (0 rad) Immediately after irradiation, these were rehydrated for 30 minutes in a water with a constant temperature of 32℃.

d. GROWING OF MV_1 GENERATION

The axillary buds in each dose were planted in wooden boxes contsining sterilized soil and grown under greenhouse conditions. The distance of the planting were 7.0 cm betweem rows and 3.0 cm between each leaf containing the bud.

The experiment consist of 50 samples per dose level with 3 replications and arranged in a randomized complete (RGB) design. In the MV_1 generation, data on the percentage of buds emerged were taken 65 days after irradiation. Seedling height, rootlenght, number of plantlets developed from bud and other morphylogical changes were recorded 155 days after irradiation. Percentage survival was reckoned 95 days after transplanting.

e. GROWING OF MV_2 GENERATION

All MV_2 plants were harvested 250 days after irradiation on individual plant basis. The bud at the base of each leaf was separated and planted in a plant-to-row plan in boxes. The distance of planting used in the MV_2 was the same with that of the MV_1. The frequency of plantlet with chlorophyll mutations and other morphological changes were obtained in the MV_2 generation. Chlorophyll mutants were selected and grown in the M_1V_3 and succeding generations. The scheme of mutation breeding in pineapple is shown as follows.

Fig. 1 Scheme of mutation breeding in pineapple

Breeding a new variety of crop takes anywhere from 12 to 15 years of intensive effort. The steps include:

Generation	Characterization axillary buds or tissue cultures treated by gamma rays.
M_1V_1	Plants grown from axillary buds
M_1V_2	Populatiion of plants grown from vegetative parts harvested from M_1V_1 Selection of desired mutants may start in this generation or later.
M_1V_3-M_1V_8	Continuing selection, genetic confirmation, multiplication and stabilization of field performance of mutant lines.
Next 2-3 generations	Comparative analyses of mutant lines during different years and in different locations.
Next 2-3 generations	Official testing before release as a new variety.

3.3 CHRYSANTHEMUM

Chrysanthemum is one of the four leading cut flowers in the world with Netherlands, South Africa, Spain and Israel being the major world producers. The current demand in Thailand for cut chrysanthemum outstrips national production. The market trend in the country suggests that in the future, new chrysanthemum varieties will be required for exotic flower sscolour, size and shape. This project aims at improving the chrysanthemum variety 'Reagan White' by *in vitro* culture in combination with induced mutation using gamma rays. Ray florets of chrysanthemum variety 'Reagan White' were cultured on MS medium supplemented with 3 concentrations of BA i.e. 1, 5 and 10 mg/l. Shoot formation was observed only on the medium supplemented with 5 and 10 mg/l BA. The shoot formation percentage was 21 and 67% for the cultures supplemented with 5 and 10 mg/l BA, respectively. By culturing ray florets on the medium supplemented with 5 mg/l BA, it took 50-60 days to produce shoots whereas it took only 21-30 days to produce shoots on the medium supplemented with 10 mg/l BA (Table II-4)

Table II-4. Percentage of shoot formation from ray florets and duration for shoot formation of chrysanthemum variety 'Reagan White' on MS medium supplemented with different concentrations of BA

Concentration of BA (mg/1)	Percentage of shoots formation from ray florets (%)	Duration for shoots formation(days)
0	0	–
1	0	–
5	21	50-60
10	67	21-30

From these results, we then used the MS medium supplemented with 10 mg/l BA to initiate the *in vitro* culture of chrysanthemum from ray florets in subsequent experiment. Multiple shoots produced were irradiated with gamma rays of 10, 20 and 30 Gy. The irradiated shoots were then transferred to fresh medium and multiplied two times from single-node cuttings. M_1V_3 shoots were rooted on half-strength MS medium and were irradiated with 10 Gy acute gamma rays. After irradiation treatment, rooted shoots were transferred to soil in the greenhouse at Mae Sa Mai Experimental Station, Chiang Mai. They were multiplied two times by conventional cutting and then transplanted in the field for observation and selection of desirable variants at flowering time. Changes in flower color, form and shape were observed in plants treated with gamma rays of 20, 30 and 40 Gy. Mutation frequency of flower form and colour mutation frequency among the irradiated plants with 20, 30 and 40 Gy was 2.9, 4.2 and 17.8%, respectively. (Table II-5).

Table II-5. Phenotype of chrysanthemum variety 'Reagan White' derived from *in vitro* irradiation with gamma rays.

Radiation dose (Gy)	No. of plants investigated	Flower form mutation frequency (%)	Colour mutation frequency(%)
10+10	68	14.7	2.9
20+10	48	22.9	4.2
30+10	28	39.3	17.8

Five promising mutants were selected - three from plants irradiated with 30 Gy and two from the plants irradiated with 40 Gy. Phenotypic characteristics of selected variants are shown in Table II-6.

Table II-6. Colour and size of selected M_1V_3 variants derived from in vitro acute irradiation of chrysanthemum variety 'Reagan White'.

Selected variants	Radiation dose(GY)	Diameter of flower(cm)	Diameter of disc florets(cm)	Length of ray florets(cm)	No. of floret whorls	Flower colour
WW_2S_7 1a	20+10	6.43±0.06	1.63±0.15	3.17±0.29	3	White
WW_2S_9	20+10	8.27±0.31	1.80±0.10	3.97±0.25	2	Yellow 2D
WW_2S_{10}	20+10	7.63±0.15	1.77±0.06	3.67±0.21	2	White
WW_3S_1	30+10	6.40±0.26	1.13±0.15	3.53±0.06	3	Yellow 2C
WW_3S_{13}	30+10	6.70±0.26	1.20±0.26	3.53±0.06	3	White

From the original white variety, mutants with attractive colour, form and size were generated. They were multiplied by *in vitro* culture (Fig. II-14) as well as by conventional cutting. They will be evaluated for their performance stability and uniformity in the field during the next planting season.

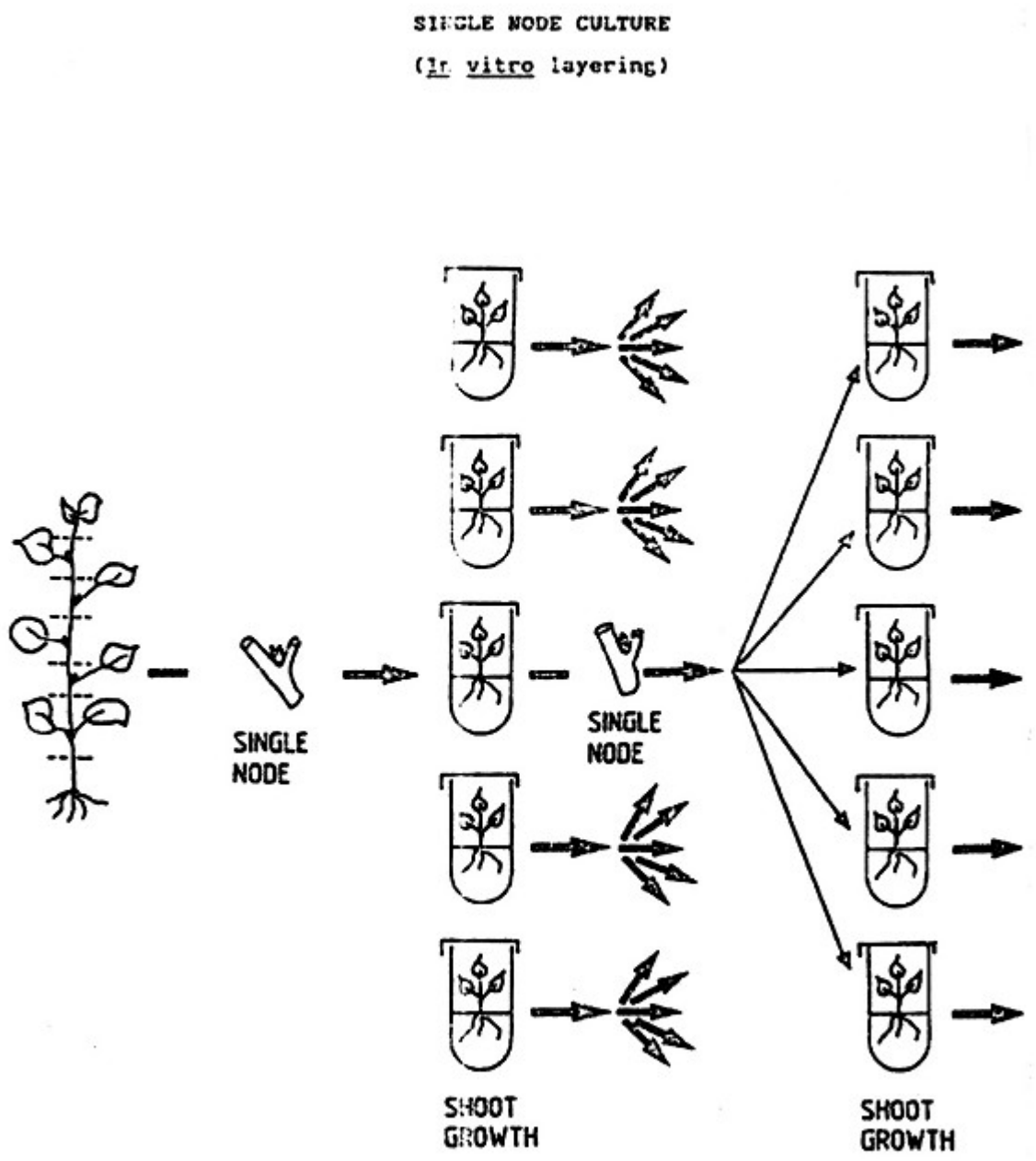


Fig. II-14 Micropropagation in Chrysanthemum

3.4 CANNA

The garden canna is a wide spread ornamental in several tropical countries especially in Thailand. In general, canna varieties arise from several hybridizations, resulting in wide variation in flower colour, shape, size and plant morphology. The scientific name of garden canna is Canna general/s. The chromosome of canna is 2n = 18 (diploid) or 3n = 27 (triploid). The former produces good seeds with high pollen fertility while the latter is virtually seedless with very low fertility. Wide diversity in canna could be achieved not only from hybridization but more rapidly from induced mutation. Gamma radiation is a good tool for improving seedless canna where the conventional crossing is ineffective. This study aims to produce new novel canna varieties from canna plants grown in the Kasetsart University Campus, by using gamma irradiation technique and selection of various characteristics such as plant morphology, flower colour, shape and size.

The initial step in the experiment was irradiation with acute gamma rays (15-25 Gy) of rhizomes and young shoots of canna. The irradiation samples were planted in pots to produce new shoots. In order to isolate mutants, progenies were observed for variation in flower colour, shape and size. When variants appeared, they were separated and grown for observation of stability and uniformity of the above characteristics. As stable mutants were obtained, they were given names and registered with the Kasetsart University' Board of Plant and Animal Varieties Registration.

Two types of variants were observed, i.e., stable and unstable. Only the stable variants could be multiplied and could give rise to new varieties. Whereas, the unstable ones tend to revert to the original parent. Ten stable mutants were obtained and were multiplied to produce new varieties. The Royal Horticulture Society colour chart was used to classify the flower colours of mutants and their controls.

Plant characteristics of mutant varieties and their controls are shown in Table II-7.

Table II-7 Some characteristics of the controls and new mutant varieties of canna from gamma rays induced mutations

Control plants	Characteristics	Radiation dose (Gy)	Mutants	Characteristics
LineNo.1	Flower deep pink(Red Group # 48A-B), medium bloom, large cluster.	20	(1) Pink Peeranuch	Flower-pale pink (Orange Group #28 C) with yellow splash, medium bloom, large cluster
LineNo.6	Flower light pink(Red Group # 49 C), medium bloom, large cluster.	15	(2) Cream Prapunpong	Flower-creamy white (Yellow Group # 2 D), medium bloom, large cluster.
Line No. 12	Flower-red (Red Group #45A-B), small bloom, large cluster; broad leaf.	20	(3) Yellow Arunee	Flower-yellow (Yellow Group # 12 A), small bloom, large clusters; broad leaf.
Line No. 24	Flower-dark orange(Orange Group 28# A-B), medium bloom, large cluster; tall plant.	25	(4) Orange Siranut	Flower-yellow-orange (Yellow-Orange Group #21 A-B), medium bloom, compact and large cluster; thick leaf and short plant.

Control plants	Characteristics	Radiation dose (Gy)	Mutants	Characteristics
Line No.10	Flower-red (Red Group # 54 A)with yellow spots(Yellow-OrangeGroup # 18 A),medium bloom, large cluster.	20	(5) Napavarn	Flower-yellow- orange (Yellow- Orange Group # 18 A) with red spots (Red Group # 37 A), medium bloom, large cluster.
		20	(6) Pink Pannee	Flower-light red(Red Group # 37A), with yellow- orange spots (Yellow-Orange Group # 18B), medium bloom, large cluster.
Line No.11	Flower-orange-red (Orange-Red Group # 32 A), small bloom, large cluster, red-green leaf.	20	(7) Orange	Flower-orange (Orange Group # 28 B), small bloom/ large cluster, green leaf,
		20	(8) Pim Ngern	Flower-orange-red (Orange-Red Group # 32 A), small bloom, large cluster; variegated leaf.
Line No.24	Flower-dark orange(Orange Group #28 B), medium bloom, large cluster.	20	(9) Wanwisa	Flower-yellow-orange (Yellow-Orange Group #14 A) with red spots, medium bloom, large cluster.
LineNo.2	Flower-red (RedGroup # 44 B) with sparse yellow streaks on petal edge, large bloom, large cluster.	20	(10) Red Ridthee	Flower-red (RedGroup # 44 B)with large yellow splash(Yellow-Orange Group #17 B), large bloom, large cluster.

PART III:

CELL AND MOLECULAR BIOTECHNOLOGY TECHNIQUES IN MUTATION BREEDING

DNA ANALYSIS ON ORCHID MUTANTS

METHOD

1) Grind plant material to a fine powder using liquid nitrogen, mortar and pestle.
2) Transfer the powder into 15ml of prewarmed (60° C) isolation buffer in a capped polypropylene tube.
3) Incubate for 30-90 min at 60°C. Mix every 10 min.
4) Add 1 vol. of chloroform-isoamyl alcohol, cap the tube and extract for 10 min on rotary shaker/by hand.
5) Centrifuge for 10 min (5000 x g, room temperature). Rcextract the aqueous phase once with fresh chloroform-isoamyl alcohol and centrifuge again.
6) Transfer final aqueous phase into a new tube. Add Rnase A to a final concentration of 100µg/ml. Mix & incubate at room temperature for 30 min.
7) Add 0.6 vol. of ice cold isopropanol, and mix gently to precipitate DNA.
8) Centrifuge for 10 min (5000 x g, 4°C)
9) Wash pellet with 20 ml of washing solution for a few minutes and collect by centrifugation (10 min,5000 x g, 4°C)
10) Invert tubes and drain on paper towel for about one hour.
11) Add appropriate TE buffer and allow the pellets to dissolve overnight (4°C) without agitation.
12) Add o.5 vol. of 7.5 M ammonium acetate solution, mix and chill on ice for 15 min.
13) Centrifuge for 30 min (10,000 x g, 4°C). Transfer supernatant to a new tube, add 2 vol. of 96% ethanol, mix by inversion, and store for 1 hour at -20°C.
14) Centrifuge for 10 min (5000 x g, 4°C), wash pellet in 70% ethanol and centrifuge again.
15) Drain final pellet, and dissolve in an appropriate volume of TE buffer.

DNA ISOLATION

A modified CTAB procedure based on the protocol on Doyle and Doyle (1987) was used to obtained good quality DNA (Weising et al. 1991).

MATERIALS AND REAGENTS

3g fresh leaf/flower
Isolation buffer: 2% CTAB(cetyltrimethylammonium bromide), 1.4 M NaCl, 20mM EDTA, 100mM Tris-HCL(pH 8.0), 0.2% β-mercaptoethanol (added just before use).
Chloroform-isoamyl alcohol (24:1)
Rnase A solution: 10mg/ml Rnase A in 10mM Tris-Hcl, 15mM Nacl (pH7.5)
100%isopropanol
Washing solution: 76% ethanol, 10mM ammonium acetate
TE Buffer: 10 mM Tris-HCL, 1 mM EDTA (PH 8.0)
7.5 M ammonium acetate

RAPD ANALYSIS

OLIGONUCLEOTIDE 10BP PRIMERS

Primer used for RAPD are: Operon Kit A & Kit B. Primers that generated bands are:
OPB-04,OPA-10
OPA-13, OPA-03

REACTION(PCR KIT)

	Volume(ul)	Final concentration
Genomic DNA	2.0	10ng/µl
Primer	2.0	20 pmol
$MgCl_2$	3.5	1.75mM
DNTPs	1.0	0.2 mM
Reaction buffer(10x)	5.0	1 x
Taq polymerase	0.5	2.5 unit
Deionised water	36.0	
Total volume:	**50.0**	

PCR PROGRAMME

Denaturation:	1min	94°C
Cycles 1-3:	30s	94°C(Denaturation)
	30s	35°C (Annealing)
	1 min 30s	72°C (Extension)
Cycles 4-38:	15s	94°C
	30s	35°C
	1 min 30s	72°C

GEL ELECTROPHORESIS

Amplified fragments were separated by electrophoresis on 2% agarose gels with a TAB running buffer and 100bp DNA ladder (Promega,USA) was used to determine the size of the fragments. Gels were stained with ethidium bromide and visualized on UV transilluminator.

METHODOLOGY FOR *IN VITRO* MUTAGENESIS OF ORCHIDS

Meanwhile, studies on the radiosensitivity of orchid protocorms showed that the results vary with time of irradiation and source of explants and also species [8]. The radiosensitivity is affected by the age of the protocorms and differences in ploidy level of different species or hybrids. Furthermore, problems in determining the optimum dose were also caused by the difficulties determining the age of the protocorms correctly and the sensitiveness of protocorms to manhandling during culturing and irradiation procedures.

Factors affecting radiosensitivity for *in vivo* plant parts must also be considered *in vitro*. In addition, evidence also indicates that concentration of auxin and/or cytokinin in the culture medium can influence the radiosensitivity of the cultured cell [9]. The medium itself when exposed to irradiation has been shown to have both stimulatory and deleterious effects on different cultured cells and tissues. High dose of irradiation may produce radiolysis products from sucrose in the medium that have effects on cell growth and differentiation [10]. To circumvent these associated problems, fresh explants and callus material can be irradiated on moist and sterile filter paper. Treated materials are then immediately transferred onto fresh non-irradiated medium.

1. Matured self-pollinated seeds from Demlrobium 'Sonia Kai' are collected.
2. Working in the laminar flow cabinet, seeds were dipped in 90% alcohol and flamed. The seeds were then left in a sterile Petri plate for a few minute.
3. Using a pair of sterile forceps and scalpel, the seed pods arc cut open longitudinally.
4. Using a new set of sterile forceps, the seeds are scooped out of the seed pod and placed on half strength macro and microelements of Murashige and Skoog (1962) medium, which is supplemented with 3% sucrose, solidified with 0.24% (wt/vol) Gelrite.
5. Cultures were maintained at 26°C with 12h photoperiod until protocorm-like bodies (PLBs) were formed.
6. Regenerated PLBs are then selected according to size and placed on wetted sterile filter paper in a Petri plate.
7. PLBs are then irradiated.
8. Irradiated PLBs are immediately transferred onto fresh 1/2 MS medium and incubated at 26°C with 12h photoperiod.
9. Irradiated PLBs were multiplied by subculturing every three weeks until four subculture cycles.

10. At the end of the fourth subculture cycle, the PLB are allowed to regenerate into whole plantlets.
11. Once the plantlets are about 1-2 cm in height and have several roots, they are transferred into a thumb pot and acclimatised in the greenhouse/shadehouse.
12. The plants are transferred into bigger pot when they reached a suitable size, and remain in this pot until mature and flower.
13. Selection for flower characteristics are done by harvesting sprays with 70% blooming flowers. Data are taken using the attached data sheet.

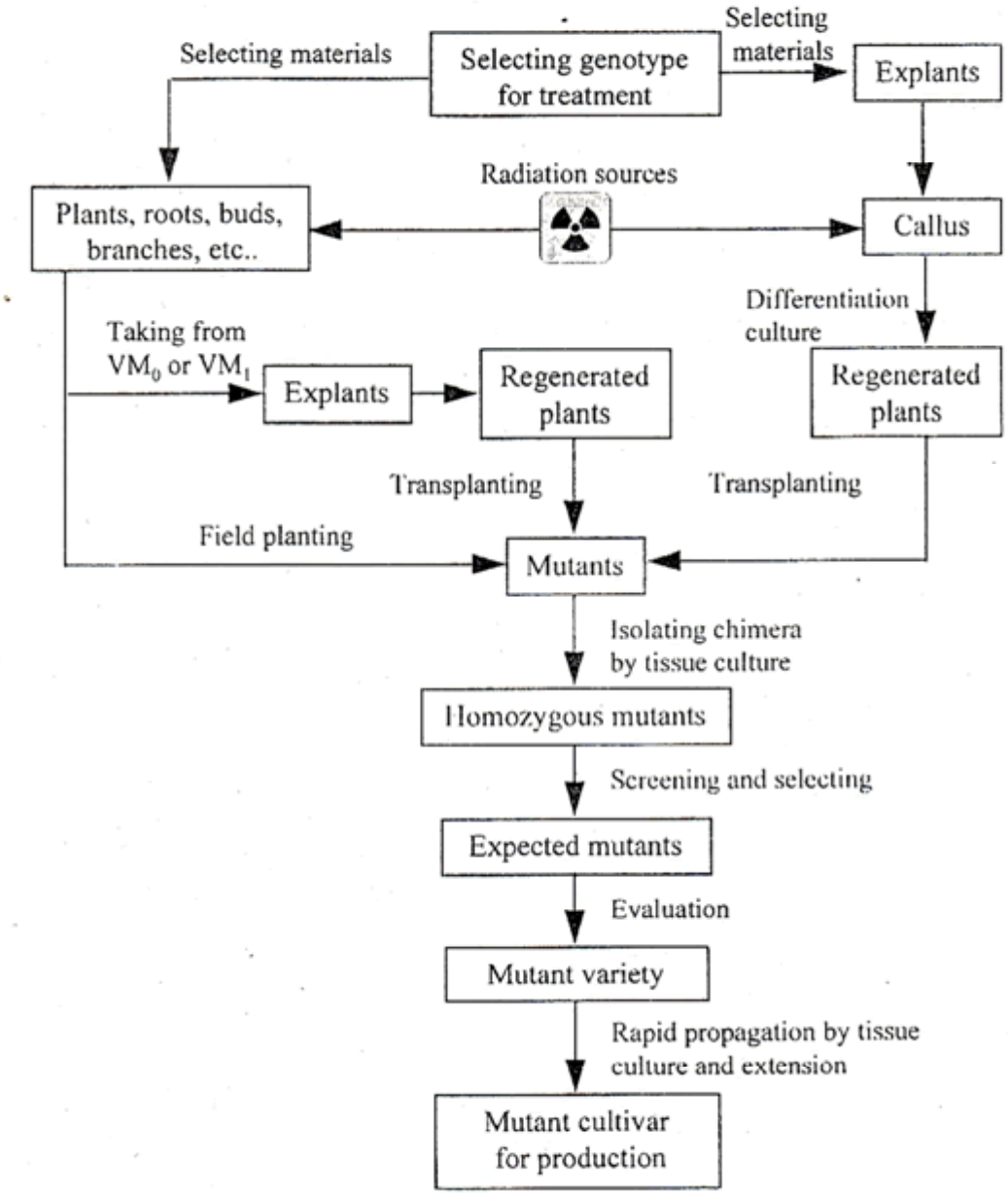
Data taken include:

Morphology of Orchid Flower
Mutant Line No.:
Species/Hybrid Name:
Locality/Origin:
Altitude:
Habitat:
Purpose:

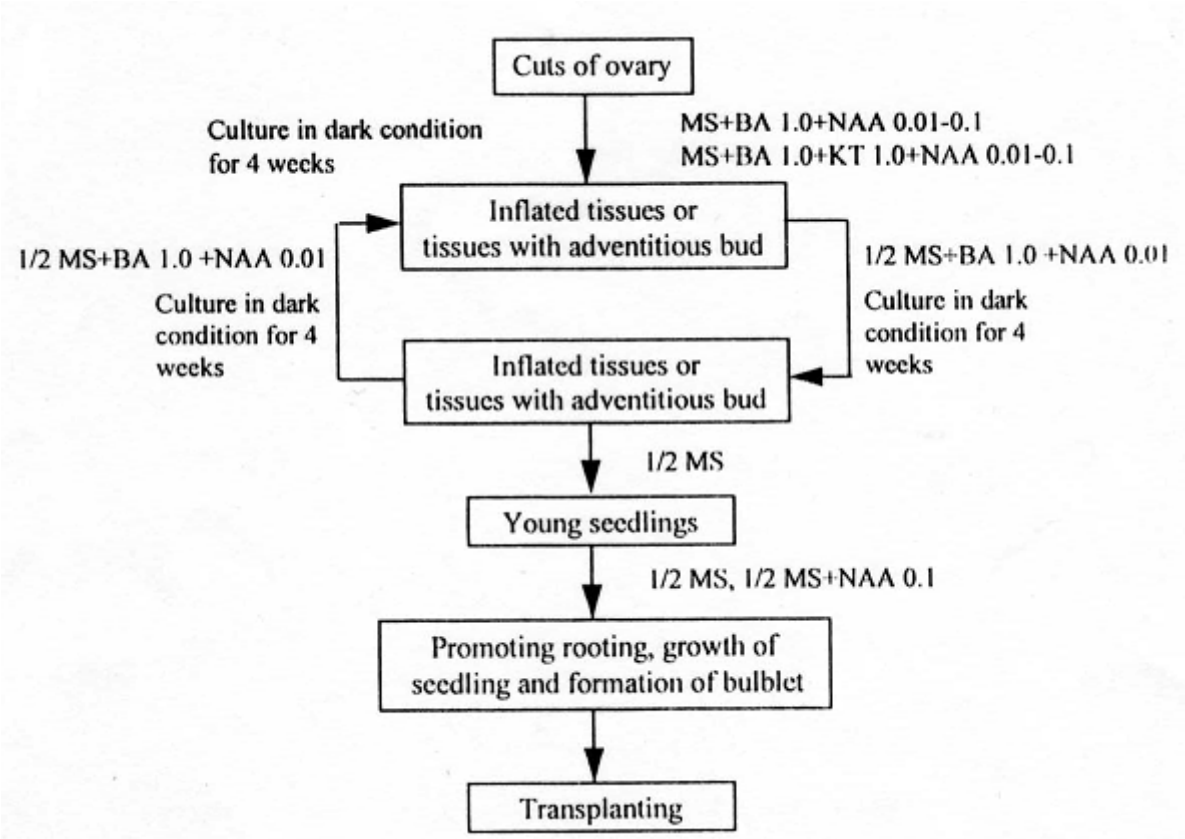
Study of Orchid Flower
Measurement and colour description

1. Horizontal measurement (spread):
2. Vertical measurement (spread):
3. Length of spray/inflorescence:
4. No. of flower and buds:
5. Length of scape (length between the base of stalk to the point where the 1st flower starts:
6. Length of pedicel:
7. Internode length:
8. Date of first flower to bloom:
9. Date of first flower to wilt:
10. Length of flower life on plant:
11. Texture of flowers
    Sepal description:
    Petal description:
12. Sepals length: Width:
    Thickness: Colour:
13. Vertical sepal length: Width:
    Thickness: Colour:
14. Petals length: Width:
    Thickness: Colour:
15. Column height: Width:
    Colour and description:
16. Lip length Width:
    Colour and description:
17. Bracts length Width:
    Colour and description:
18. The position/no, of flower the first to wilt:
19. No. of flowers and buds at time of wilts:
20. Shelflifc (harvesting at 70%) flowering
    Date of 1st flower
    Date of 70% flowering
    Date when the 1st flower wilts
    Shelflife
21. In florescence/flower stalk - erect/pendulous
22. Brief description of the flowers:

BREEDING TECHNIQUES OF MUTATION INDUCTION COMBINED WITH TISSUE CULTURE FOR CHRYSANTHEMUM

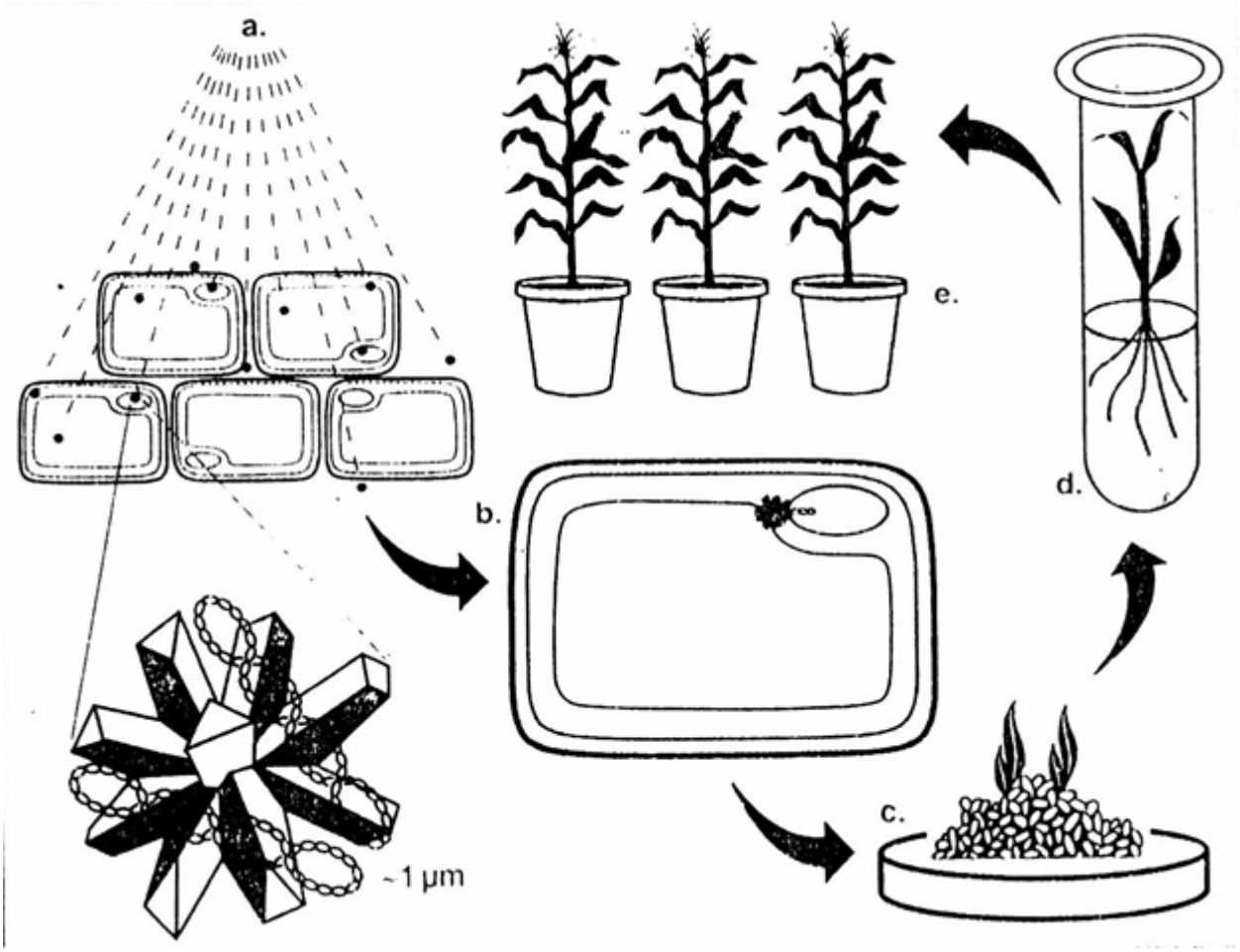


PRODUCER OF RAPID PROPAGATION WITH TISSUE CULTURE FOR OVARY OF GLADIOLUS GANDAVENSIS

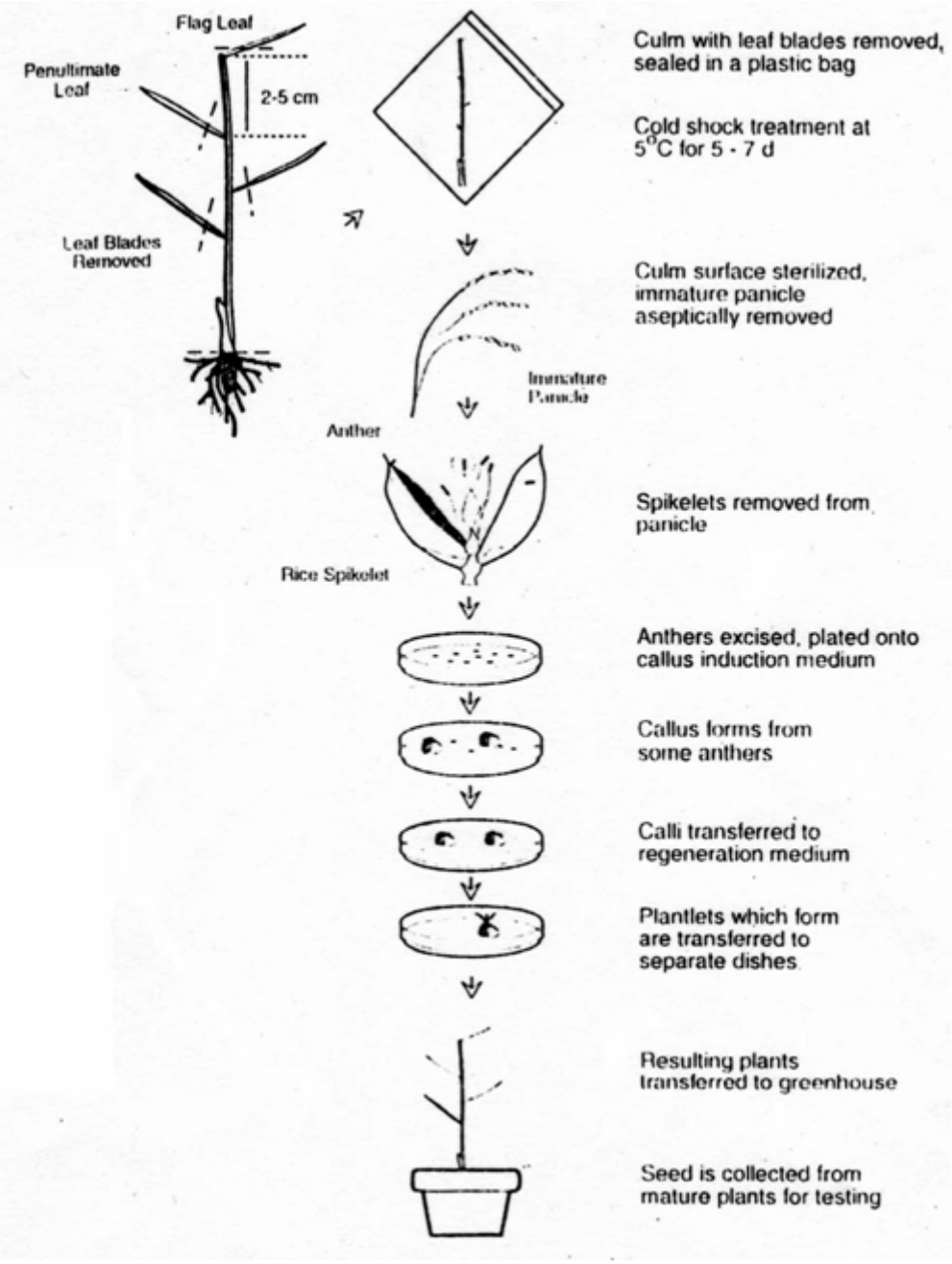


MICROPROJECTILE BOMBARDMENT FOR MAIZE TRANSFORMATION

a:
Particle accelerates DNA-coated tungsten microprojectiles at high speed into plant cells such as embryogenic callus. Inset shows the supercoiled DNA in the tungsten particle.
b:
DNA-coated microprojectiles are accelerated into a large number of plant cells where DNA migrates off the particles, and for a small proportion of cells the vector DNA integrates into plant chromosomal DNA.
c:
Transgenic cells with integrated vector DNA divide and can regenerate plants in the presence of a selective agent such as Bialaphos, while nontransgenic cells do not grow or regenerate plants.
d:
Regenerated transgenic plants produce roots in the presence of Bialaphos in preparation for transfer to greenhouse growth, while nontransgenic plants will not produce roots.
e:
Transgenic plants are grown to maturity in the greenhouse and are wither selfed or crossed to obtain seed.



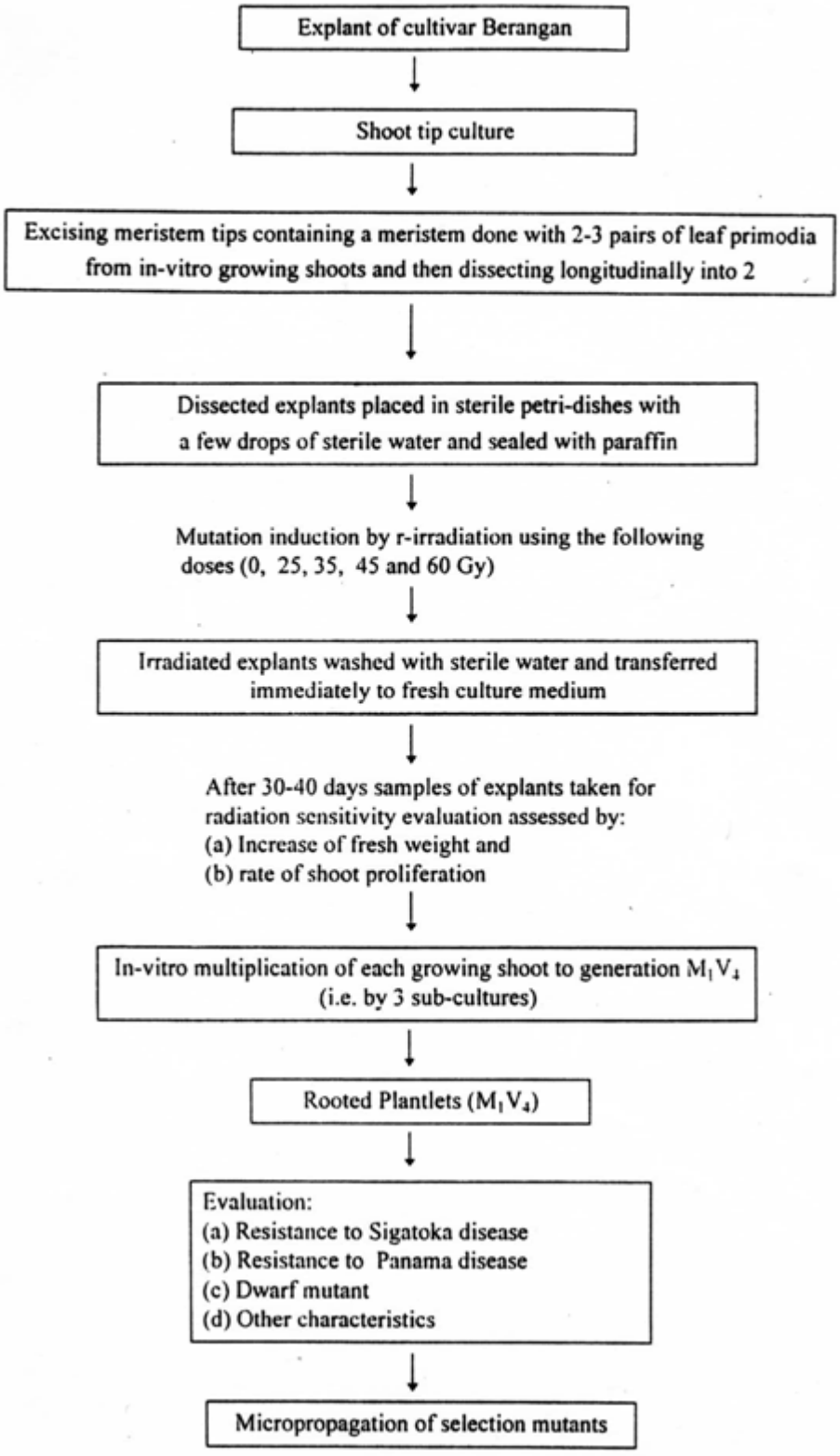
ANOTHER CULTURE OF RICE



Steps involved in another culture of rice. (Graphic by Deren Hansen)

Research strategies of the whole procedures starting with the *in vitro* shoot tip cultures and evaluation is provided with the schematic representations.

Research Strategies (Schematic representation)



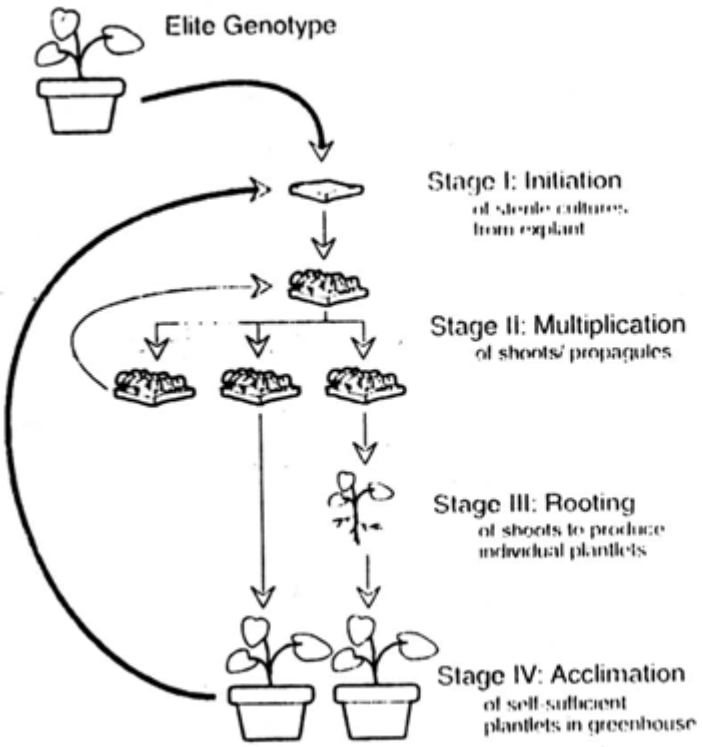


Fig. The four stages of a typical micropropagation system. *Stage II*, multiplication, can be repeated as often as needed. *Stage III*, rooting, can be an optional step, depending on the species. (Graphic by Deren Hansen)