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## Application of mutation in crop improvement

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### Abstract

Genetic variation is a source of phenotypic diversity and is a major driver of evolutionary diversification. Heritable variation was observed and used thousands of years ago in the domestication of plants and animals. The mechanisms that govern the inheritance of traits were later described by Mendel. Plant breeding requires genetic variation of useful traits for crop improvement. The induction of mutations has been used to enhance the yield, better nutritional quality and wider adaptability of world's most important crops such as wheat, rice, pulses, millets and oilseeds. The total area covered by commercially released mutant cultivars clearly indicates that they have played a significant role in solving food and nutritional security problems in many countries. Of all the mutant varieties developed, majority of mutants were produced through direct mutagenesis of the plant propagules and also there are several reports of mutants derived by irradiating rooted stem cuttings, which paves the way for in vitro mutagenesis. The incorporation of desired traits from non-adapted landraces or crop wild resources can speed up crop improvement. Among the different strategies to enhance crop improvement programs, induced mutagenesis has contributed immensely by creating mutant varieties with improved and desirable genetic changes in agronomically important traits of the crop plants. Such genetic changes can occur spontaneously naturally at a very low rate or experimentally induced by physical and chemical mutagens. Conventional mutation techniques have often been used to improve yield, quality, and disease and pest resistance in crops or to increase the attractiveness of flowers and ornamental plants. In general, mutation is the main source of genetic variation, which is the raw material for evolution by natural selection. Recognize that mutations are the basis of microevolution and that adaptations enhance the survival and reproduction of individuals in a population. Mutation breeding has greater impact in sustainable crop production by developing new mutant varieties. With the advances in genomics research and availability of genome sequences, induced mutants continue to be a genetic resource for elucidating genetic mechanisms and metabolic pathways. Agricultural sustainability and food security are major challenges facing continued population growth. Integration of existing and new technologies for the induction and exploitation of genetic diversity towards developing healthier, nutritious and productive crops is the need of the hour. Mutagenesis is a proven technology for the development of improved or novel varieties with desirable traits. Several mutant genes have been successfully explored, either directly or indirectly, to complement crop productivity.

**Keywords:** Mutation, genetic variation, induced mutation, mutagenesis, transgenic crop

### Introduction

Mutation is the alteration in the genetic material of a cell of a living organism or of a virus that is more or less permanent and that can be transmitted to the cell's or the virus's descendants. The genomes of organisms are all composed of DNA whereas viral genomes can be of DNA or RNA. Mutation refers to sudden heritable change in the phenotype of an individual. The term mutation was originally coined by Dutch botanist Hugo De Vries since 1848–1935 (Jain, 2010a) [4]. Mutation breeding is defined as the deliberate induction and development of mutant lines for crop improvement. Mutation is the most commonly used method in asexually propagated crops and self-pollinated crops for creation of variation that used for improvement. Induced mutations are considered as an alternative to naturally occurring variation as the source of germplasm for plant improvement programs and as an alternative to hybridization and recombination in plant breeding.

Mutation is a heritable change in a genetic characteristic of an organism and is a natural process that creates new variants (alleles) of genes. Mutation is the primary source of all genetic variations existing in any organism, including plants. Variation so created by mutation provides the raw material for natural selection and is a driving force in evolution. The standard technique of creating variability by means of altering genes through induction of mutations by physical or

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chemical mutagens and using the same effectively through elaborate methods of selection techniques in various generations for improvement of a particular crop species for desired objectives is called mutation breeding. This is frequently practiced by plant breeders all over the world for crop improvement.

The genetic improvement of crops is a crucial component of the efforts to address pressures on global food security and nutrition (Ronald, 2011) <sup>[16]</sup>. It is estimated that food production should be at least doubled by the year 2050 in order to meet the needs of a continually growing population (Ray *et al.*, 2013) <sup>[15]</sup>. The availability of heritable variation is a prerequisite for genetic improvement of crops. Where sufficient variation does not exist naturally, it can be created through either random or targeted processes.

Globally, the current human population is increasing day to day and expected to reach 9 billion by 2050 and that will lead to food scarcity on earth. To overcome this increasing demand for food and proper nourishment, an improvement in food production is urgently needed (Ronald, 2014) <sup>[17]</sup>. The envisaged in food production is daunting because of limited available arable land, depleting water resource and varying climatic condition. There are different mechanisms for harnessing the heritable variations encoded in the genetic makeup of existing crop plants so as to use them in the crop improvement programs. The incorporation of desired traits from non-adapted landraces or crop wild resources can speed up crop improvement. Putative parental material can also be induced to mutate so as to obtain new genes that control desired traits for new crop variety development (Suprasanna *et al.*, 2009) <sup>[23]</sup>.

Among the different strategies to enhance crop improvement programs, induced mutagenesis has contributed immensely by creating mutant varieties with improved and desirable genetic changes in agronomically important traits of the crop plants. Mutagenesis has become more efficient in combination with advanced molecular biology techniques and in vitro culture methods that result in enhancement of crop improvement or breeding program particularly under the global climate change (Jain, 2010a) <sup>[10]</sup>. Such induced mutagenesis also helps in the mining of new gene alleles that do not occur in the germplasm (Roychowdhury and Tah, 2013) <sup>[18]</sup>. Mutation is the ultimate source of all genetic changes which provide the raw material for evolution and it is a valuable approach for improvement of economic characters of plants. Such genetic changes can occur spontaneously naturally at a very low rate or experimentally induced by physical and chemical mutagens (Mba *et al.*, 2007). The use of diverse genetic resources is important for breeding crop varieties (Glaszmann *et al.*, 2010) <sup>[3]</sup>.

Crop species with narrow genetic diversity are susceptible to emerging pathogens or other constraints leading to loss of productivity and this may lead to a serious decline in the areas of adaptation (Dyer *et al.*, 2014) <sup>[1]</sup>. Genetic variability of crops can be partitioned into variability between crop cultivars and variability within a crop cultivar (the genetic differences within the population of plants that make up the cultivar). The extent of variation between cultivars is a function of the number that makes up a significant part of the crop acreage and of the genetic differences between them. The largest diversity of the crop germplasm provides greater opportunities for improvement regarding its environmental adaptability and acquiring better agronomic traits from the crop species. Genetic diversity of plants determines their potential for improved efficiency and hence their use for breeding, which eventually may result in enhanced food production. The success of breeding program

depends to a large measure on the degree of genetic divergence. Genetic diversity is a key factor for crop improvement.

Mutation is the origin of all new genetic diversity, occurring when there are occasional errors in the replication of DNA or other elements of the production and packaging of genetic information within the cells. Although it implies something negative, mutations can have positive, neutral, or deleterious impacts. Mutations occur rather slowly but continuously. Mutations at one level, for example, in the nucleotides that are the basis of DNA may not all be expressed at other levels such as protein differences or observable changes in the appearance of a plant. The rate of mutation is useful in determining evolutionary relationships. Mutation breeding is a fundamental and highly successful tool in the global efforts of agriculture to feed an ever increasing and nutritionally demanding human population. Plant mutation breeding is the process of exposing plant seeds, cuttings or cell cultures to radiation, such as gamma rays and then planting the seed or cultivating the irradiated material in a sterile medium that generates a plantlet.

Mutations are the major source of genetic variability and artificial mutations can be induced by mutagens (Oladosu *et al.*, 2016) <sup>[13]</sup>. There are three major tools for mutagenesis: biological agents such as transposons, retrotransposons, and T-DNA; physical agents such as ionizing radiations and chemical agents such as alkylating agents; and azides (Serrat *et al.*, 2014) <sup>[19]</sup>. Mutation breeding has resulted in thousands of improved varieties with higher yields and improved tolerance to pests, diseases and environmental stresses. The genetic diversity of crop plants is the foundation for the sustainable development of improved crop varieties for meeting present and future food security challenges. Induced mutations offer numerous benefits to crop improvement, especially when conventional breeding techniques fail for the lack of appropriate genetic variation.

Sudden, heritable changes in the genetic material, DNA, are known as mutations. Selection of naturally occurring mutations in wild, ancestral species helped humans in the domestication and further improvement of today's crop plants. Plant breeding based on the science of genetics, as practiced over the past 100 years, exploited the available genetic variability in the primary gene pool of crop plants and sometimes in related species. This approach enlarged the yield potential of crops several fold. It also a) improved the stability of yield by incorporating resistance to various biotic and abiotic stresses; b) improved quality of the produce; and c) altered the adaptability of crop species, providing opportunities to grow new crops for food security outside their traditional range. Genetically improved seed (other planting material) is the most significant input for developing sustainable cropping systems for food security and economic growth. Half of the increased productivity of today's crop plants comes from genetic improvements. The other half is contributed by inputs and management practices.

A mutation is the original source of genetic variation and primarily creates genetic diversity and can have positive, neutral and negative impacts in genetic alteration of crop species. Mutation is the sudden heritable changes of genetic diversity occurred occasionally through aberration of genetic materials like DNA, RNA and protein within the cells. Mutation has a great role in increasing genetic diversity in order to feed the increasing human population (Smith BD, 1989) <sup>[22]</sup>. Mutation is primarily causes the alteration of genetic diversity. Genetic diversity is the variation occurred in genetic information, which depends on frequency and diversity of alleles among individuals within a population or a species. Mutation is the driving force for sustainable genetic diversity creation which uses in further

improvement. Induced mutageneses are broadening the genetic variation whereas a conventional breeding approach is narrowing genetic variability for improvement for long period of time. The utilization of mutation breeding is significantly generating crop genetic diversities through improvement of crops to improve the livelihood of the communities (Oladosu Y *et al.*, 2016) <sup>[13]</sup>. The objective of the paper was to understand the significance mutation in crop improvement to feed the world growing population.

## 2. Role of Mutation in Crop Improvement

It is well known that evolution and practical breeding depend on genetic variation. The variations that are found in nature do not represent the original spectra of spontaneous mutations. Rather, they are the result of genotypes recombining within populations and their continuous interaction with environmental factors (Novak FJ and Brunner H, 1992) <sup>[12]</sup>. Green plants are essential for human existence as a source of food, clothing and energy resources. Mutations are the primary source of all genetic variations existing in any organism, including plants (Kharkwal MC, 2012) <sup>[6]</sup>. The resulting variation provides the raw material for natural selection and is also a driving force in evolution. Spontaneous mutations are very rare and random in terms of time of occurrence, which makes them more difficult to use in plant breeding programmes (Lonnig WE, 2005) <sup>[9]</sup>. In this way, mutant forms showing both large and small effects on the phenotype arise for all kinds of traits (Kharkwal MC, 2012) <sup>[6]</sup>. Mutation breeding involves the development of new varieties by generating and utilizing genetic variability through chemical and physical mutagenesis. It is now a pillar of modern plant breeding, along with recombinant breeding and transgenic breeding (Shu QY *et al.*, 2012) <sup>[2]</sup>.

Mutagenesis is the process whereby sudden heritable changes occur in the genetic information of an organism not caused by genetic segregation or genetic recombination, but induced by chemical, physical or biological agents (Roychowdhury R and Tah J, 2013) <sup>[18]</sup>. Mutation breeding employs three types of mutagenesis. These are induced mutagenesis, in which mutations occur as a result of irradiation (gamma rays, X-rays, ion beam, etc.) or treatment with chemical mutagens; site-directed mutagenesis, which is the process of creating a mutation at a defined site in a DNA molecule; and insertion mutagenesis, which is due to DNA insertions, either through genetic transformation and insertion of T-DNA or activation of transposable elements (Forster BP and Shu QY, 2012) <sup>[20]</sup>. Plant breeding requires genetic variation of useful traits for crop improvements (Novak FJ and Brunner H, 1992) <sup>[12]</sup>. However, multiple mutant alleles are the sources of genetic diversity for crop breeding as well as functional analysis of the targeted gene in many cases. The key point in mutation breeding is the process of identifying individuals with a target mutation, which involves two major steps: mutant screening and mutant confirmation (Forster BP and Shu QY, 2012) <sup>[20]</sup>. Mutant screening is a process involving selection of individuals from a large mutated population that meet specific selection criteria, e.g. early flowering, disease resistance as compared to the parent. However, these selections are often regarded as putative mutants or false mutants. Mutant confirmation, on the other hand, is the process of re-evaluating the putative mutants under a controlled and replicated environment using large samples. Through this process, many putative mutants are revealed to be false mutants. In general, the mutations that are important in crop improvement usually involve single bases and may or may not affect protein synthesis (Mba C, 2013).

Mutation breeding and plant mutagenesis play a significant role in increasing the genetic variability for desired traits in various food crops (Kozgar, M.I *et al.*, 2012) <sup>[7]</sup>. Induced mutagenesis is one of the most efficient tools used for the identification of key regulatory genes and molecular mechanisms. It is a promising approach to develop new varieties with improved agronomic characteristics, such as higher stress tolerance potential (biotic and abiotic stress) and bio-fortification. Additionally, various mutagenesis approaches have been used to study the evolutionary relationship as well as for the genetic improvement of many organisms, including microbes, animals, and plants (Sikora, P *et al.*, 2011) <sup>[21]</sup>. Technological advances in molecular biology have re-augmented the mutation breeding making it more efficient than ever thought before.

The first step in plant breeding is to identify suitable genotypes containing the desired genes among existing varieties, or to create one if it is not found in nature. In nature, variation occurs mainly as a result of mutations and without it and plant breeding would be impossible without variation. In this context, the major aim in mutation-based breeding is to develop and improve well-adapted plant varieties by modifying one or two major traits to increase their productivity or quality. Both physical and chemical mutagenesis is used in inducing mutations in seeds and other planting materials. Then, selection for agronomic traits is done in the first generation, whereby most mutant lines may be discarded. Mutation breeding sometimes referred to as variation breeding.

The utilization of induced mutants in crop improvement is called mutation breeding. Mutation may be the change in gene, chromosome or plasma gene (genetic material inside mitochondria and chloroplasts). The mutation produced by change in the base sequence of gene is called point mutation or gene mutation. The concept of induced mutagenesis for crop improvement developed dated back to the beginning of 20th century. During the past 80 years, mutation breeding has been successfully utilized for the improvement of crops as well as to supplement the efforts made using traditional methods of plant breeding. There are two major types of Mutation:

### 2.1 Spontaneous Mutation

Spontaneous mutations arise from a variety of sources including errors in DNA replication, spontaneous lesions and transposable genetic elements. Mutation occurs in nature are called spontaneous mutation. Spontaneous mutation occurs in the organism without any treatment at low rate in the nature. The frequency of spontaneous mutation is  $10^{-6}$  (one in one in million). Different genes in an organism show different mutation rate. Rates of spontaneous mutation per genome as measured in the laboratory are remarkably similar within broad groups of organisms but differ strikingly among groups. Mutation is one of the basic phenomena of life. Without mutation, the gradual development of life from inorganic material would have been impossible and the evolution of living beings from the first groups of molecules in which a primitive, information-carrying unit cooperated with an energy gaining device up to the present diversity of highly refined living organisms could not have occurred. Spontaneous mutations are alleles of initially unknown genes and are given allele names and symbols based on their phenotype. Spontaneous mutations and deletions can be used to identify important functional regions within a gene. Spontaneous mutations are the result of errors in natural biological processes, while induced mutations are due to agents in the environment that cause changes in DNA structure.

## 2.2 Induced Mutation

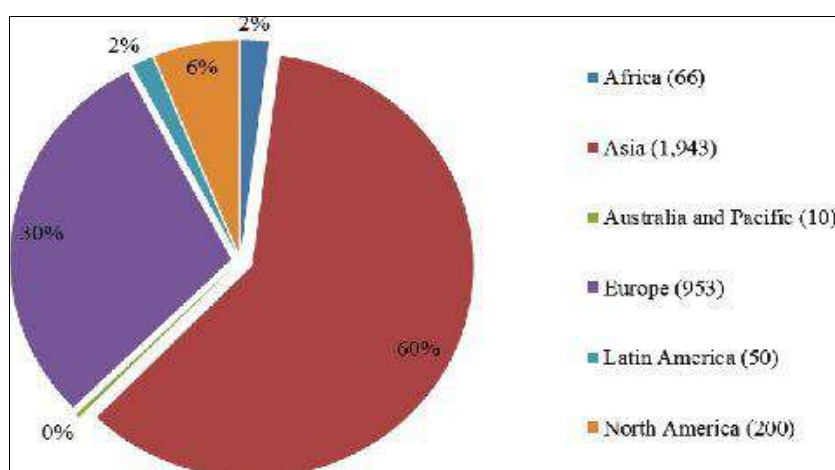
Induced mutations are very effective in creating genetic variability for various economic characters in crop plants. Induced mutations have been used for increasing the range of genetic variability in barley, oats, wheat and many other crops. In asexually propagated crops like sugarcane and potato, somatic mutations may be useful, because the mutant plant can be multiplied as a clone. Mutations induced in an organism by treatment with physical or chemical mutagen are called induced mutations. The agents which are used to induce mutation are called mutagens. Certain genes in an organism promote the mutation of other genes nearby in the chromosomes. Induced mutations are useful in crop improvement in several principal ways, viz: Development of improved varieties, induction of

male sterility, production of haploids, creation of genetic variability and overcoming self-incompatibility.

Mutations are induced by physical (gamma radiation, high and low energy beams) and chemical (ethyl methane sulfonate, EMS) mutagen treatment of both seed and vegetative propagated crops. The mechanism of mutation induction is that the mutagen treatment breaks the nuclear DNA and during the process of DNA repair mechanism, new mutations occur randomly and are heritable. The changes can also occur in cytoplasmic organelles and also results in chlorophyll mutations, chromosomal or genomic mutations that enable plant breeders to select useful mutants such as abiotic and biotic stresses and others resistant trait (Mohan Jain, S and Suprasanna, P, 2011) <sup>[11]</sup>.

**Table 1:** Classification and Brief Description of Mutation

Basis of Classification and Type of Mutation	Brief Description
<b>1. Based on source</b>	
Spontaneous	Mutation that occur in nature
Induced	Mutation which are produced by the use of mutagenic agent
<b>2. Based on direction</b>	
Forward mutation	Any change from wild type allele
Reverse mutation	Any change from mutant allele to wild type
<b>3. Based on tissue</b>	
Somatic mutation	A mutation in somatic tissue
Germinal mutation	A mutation in germ line cell
<b>4. Based on survival</b>	
Lethal	A mutation which kills the individual that carries it
Sub-lethal	When mortality is more than 50% of the individuals that carry mutation
Sub-vital	When mortality is less than 50% of the individuals that carry mutation
Vital	When all mutant individuals survive
<b>5. Based on site</b>	
Nuclear mutation	A mutation in nuclear gene
Cytoplasm mutation	A mutation in cytoplasm gene
<b>6. Based on character</b>	
Morphological	A mutation that alters the morphological characters of individuals
Biochemical	A mutation that alters biochemical function of individuals
<b>7. Based on visibility</b>	
Micro-mutation	Mutation with invisible phenotypic changes. Generally observed in quantitative characters
Macro-mutation	Mutation with distinct morphological changes in phenotypes. Generally found in qualitative characters



Source: Joint Division of the Food and Agriculture Organization of the United Nations and the International Atomic Energy Agency

**Fig 1:** Distribution of mutant crop varieties by continents (Accessed on 15th July, 2015).

## 2.3 Mutagenic Agents

Agents of artificial mutations are called mutagens. They are generally grouped into two broad categories, namely chemical mutagens and physical mutagens

### 2.3.1 Physical Mutagenesis

In the past 80 years, physical mutagens, mostly ionizing radiations, have been used widely for inducing hereditary aberrations and more than 70% of mutant varieties were developed using physical mutagenesis. Radiation is defined as



energy travelling through a distance in the form of waves or particles. These are relatively high-energy levels of electromagnetic (EM) spectrum that are capable of dislodging electrons from the nuclear orbits of the atoms that they impact upon. The impacted atoms, therefore, become ions. Hence, the term ionizing radiation. These ionizing components of the EM include cosmic, gamma ( $\gamma$ ) and X-rays. The most commonly used physical mutagens are shown in Table 1. X-rays were the first to be used to induce mutations. Since then, various subatomic particles (neutrons, protons, beta particles and alpha particles) have been generated using nuclear reactors Wiley-Blackwell, (2006). Gamma radiation from radioactive cobalt ( $^{60}\text{Co}$ ) is widely used. It has high penetrating potential and is hazardous. However, it can be used for irradiating whole plants and delicate materials, such as pollen grains. Various mutants have been developed through gamma radiation. The mutagenic effect results mostly from DNA double-strand breaks.

The mutants show higher potential for improving plant architecture leading to better crop improvement and are used as

a complementary tool in plant breeding. Gamma rays have a shorter wave length and therefore, possess more energy than protons and X-rays, which gives them ability to penetrate deeper into the tissue. Neutrons are hazardous and hence have less penetrating abilities, but they are known to cause serious damage to the chromosomes. They are best used for materials, such as dry seeds. Various forms of neutrons were also studied extensively for their use in mutagenesis in the 1960s and 1970s. Though it has been proved to be an effective mutagen, particularly for producing large DNA fragment deletions, the application of neutrons in induced mutagenesis is limited. The mutagenic effect of ultraviolet light was discovered by Altenburg through irradiation of the polar cap cells of fruit fly eggs. The mutagenic potential of these rays have since been confirmed in many organisms. In those organisms, germ tissue could be easily exposed to the low-penetrating ultraviolet light which resulted in covalent dimerization of adjacent pyrimidine (Oladosu, Y *et al.*, 2015) <sup>[14]</sup>

**Table 2:** Examples of commonly used physical mutagens

Mutagen	Source	Characteristics	Hazard
X-rays	X-ray machine	Electromagnetic radiation; penetrates tissues from a few millimeters to many centimeters	Dangerous, penetrating
Gamma rays	Radioisotopes and nuclear reaction	Electromagnetic radiation produced by radioisotopes and nuclear reactors; very penetrating into tissues; sources are $^{60}\text{Co}$ (Cobalt-60) and $^{137}\text{Cs}$ (Caesium-137)	Dangerous, very penetrating
Neutrons	Nuclear reactors or accelerators	There are different types (fast, slow, thermal); produced in nuclear reactors; uncharged particles; penetrate tissues to many centimeters; source is $^{235}\text{U}$	Very hazardous
Beta particles	Radioactive isotopes or accelerators	Produced in particle accelerators or from radioisotopes; are electrons; ionize; shallowly penetrating; sources include $^{32}\text{P}$ and $^{14}\text{C}$	May be dangerous
Alpha particles	Radioisotopes	Derived from radioisotopes; a helium nucleus capable of heavy ionization; very shallowly penetrating	Very dangerous
Protons	Nuclear reactors or accelerators	Produced in nuclear reactors and accelerators; derived from hydrogen nucleus; penetrate tissues up to several centimeters	Very dangerous
Ion beam	Particle accelerators	Produced positively charged ions are accelerated at a high speed (around 20%–80% of the speed of light) deposit high energy on a target	Dangerous

Source: Oladosu, Yusuff *et al.* (2015) <sup>[14]</sup>

### 2.3.2 Chemical mutagenesis

The effect of chemical mutagens on plant materials is generally considered milder. An advantage of chemical mutagenic agents is that they can be applied without complicated equipment or facilities. The ratio of mutational to undesirable modifications is generally higher for chemical mutagens than for physical. Usually, the material is soaked in a solution of the mutagen to induce mutations. However, chemical mutagens are generally carcinogenic and therefore, extra care must be taken for health protection during the procedure. Material and safety data sheets for the specific chemical mutagen chosen should be carefully read and the agent should be appropriately inactivated before disposal. Despite the large number of mutagenic compounds, only a small number has been tested in plants.

Among them, only a very restricted group of alkylating agents has found large application in plant experimental mutagenesis and plant mutation breeding. Over 80% of the registered new mutant plant varieties reported in the International Atomic Energy Association (IAEA) database obtained via chemical mutagenesis were induced by alkylating agents. Of these, three compounds are significant: ethyl methane sulphonate (EMS), 1-methyl-1-nitrosourea and 1-ethyl-1-nitrosourea, which account for 64% of these varieties. (Javed, I *et al.* 2016) <sup>[5]</sup> Alkylating agents can be found among a large array of classes of compounds, including Sulphur mustards, nitrogen mustards,

epoxides, ethylene imines, ethylene imides, alkyl methanesulfonate, alkyl nitrosoureas, alkyl nitrosoamines, alkyl nitrosoamides, alkyl halides, alkyl sulphates, alkyl phosphates, chloroethyl sulphides, chloroethylamines, and diazoalkanes. One of the most effective chemical mutagenic groups is the group of alkylating agents (these react with the DNA by alkylating the phosphate groups as well as the purines and pyrimidine. Another group is that of the base analogues (they are closely related to the DNA bases and can be wrongly incorporated during replication).

### 2.4 Genetic Transformation of Plants (T-DNA) or Insertion Mutation

For more than two decades, scientists have used Agrobacterium-mediated genetic transformation to generate transgenic plants. An initial technology to introduce genes of interest (goi) into Agrobacterium seems complex microbial genetic methodologies that inserted this gene of interest into the transfer DNA (T-DNA) region of large tumor-inducing plasmids (Ti-plasmids). However, scientists eventually learned that T-DNA transfer could still be effected if the T-DNA region and the virulence (*vir*) genes required for T-DNA processing and transfer was split into two replicons. This binary system permitted facile manipulation of Agrobacterium and opened up the field of plant genetic engineering to numerous laboratories (Lee, L.Y and

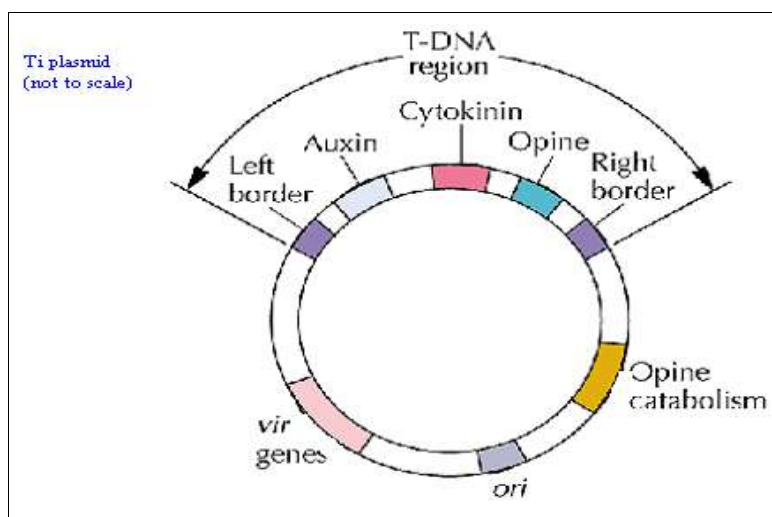
Gelvin, S.B., 2008) [8]. Transfer requires three major elements: (1) T-DNA border repeat sequences (25 bp) that flank the T-DNA in direct orientation and delineate the region that will be processed from the Ti/Ri-plasmid (Yadav *et al.*, 1982) [24]; (2) *vir* genes located on the Ti/Ri-plasmid; and (3) various genes (chromosomal virulence [*chv*] and other genes) located on the bacterial chromosomes. These chromosomal genes generally are involved in bacterial exopolysaccharide synthesis, maturation, and secretion.

The *vir* region consists of approximately 10 operons (depending upon the Ti- or Ri-plasmid) that serve four major functions: 1) Sensing plant phenolic compounds and transducing this signal to induce expression of *vir* genes (*virA* and *virG*). VirA and VirG compose a two-component system that responds to particular phenolic compounds produced by wounded plant cells. Because wounding is important for efficient plant transformation, *Agrobacterium* can sense a wounded potential host by perceiving these phenolic compounds. Activation of VirA by these phenolic inducers initiates a phospho-relay, ultimately resulting in phosphorylation and activation of the VirG protein. Activated VirG binds to the *vir* box sequences preceding each *vir* gene operon, allowing increased expression of each of these operons. In addition to induction of the *vir* genes by phenolics, many sugars serve as co-inducers. These sugars are perceived by a protein, ChvE, encoded by a gene on the *Agrobacterium*

chromosome. In the presence of these sugars, *vir* genes are more fully induced at lower phenolic concentrations.

(2) Processing T-DNA from the parental Ti- or Ri-plasmid (*virD1* and *virD2*). Together, VirD1 (a helicase) and VirD2 (an endonuclease) bind to and nick DNA at 25-bp directly repeated T-DNA border repeat sequences). The VirD2 protein covalently links to the 5' end of the processed single-strand DNA (the T-strand) and leads it out of the bacterium, into the plant cell, and to the plant nucleus. (3) Secreting T-DNA and Vir proteins from the bacterium via a type IV secretion system (*virB* operon and *virD4*). The *Agrobacterium virB* operon contains 11 genes, most of which form a pore through the bacterial membrane for the transfer of Vir proteins. Currently, we know of five such proteins that are secreted through this apparatus: VirD2 (unattached or attached to the T-strand), VirD5, VirE2, VirE3, and VirF. VirD4 acts as a coupling factor to link VirD2-T-strand to the type IV secretion apparatus.

(4) Participating in events within the host cell involving T-DNA cytoplasmic trafficking, nuclear targeting, and integration into the host genome (*virD2*, *virD5*, *virE2*, *virE3*, and *virF*). VirD2 and VirE2 may play roles in targeting the T-strand to the nucleus; In addition, VirE2 likely protects T-strands from nucleolytic degradation in the plant cell. VirF may play a role in stripping proteins off the T-strand prior to T-DNA integration (Tzfira *et al.*, 2004).



Source: Genetic Transformation of Plants manual notes

## 2.5 Impact of induced mutation

The Plant Breeding and Genetics Section of the Joint FAO/IAEA Division maintain a Mutant Variety Database (MVD) which lists more than 2250 cultivars of 175 species that have been officially released in sixty countries. Among these, 1700 are in the food crops cereals (1072), legumes (311), vegetables (66), and oil crops (59); the rest are industrial crops and ornamental plants<sup>1</sup>. In fact, the MVD does not reveal the full usage of the induced variability because it does not include all the derivatives selected after cross breeding. The impact of the new cultivars can only be estimated by the acreage in which they are grown. In the absence of well-organized seed production in most developing countries, and given the prevalence of informal seed sales between farmers, it is very difficult to estimate the real spread of a given derivative. To take one important example, rice is the staple food for nearly half of the human population. More than 90% is grown in developing countries

### 2.5.1 Economic impact of a new mutant variety

Area planted to the variety and percentage of the area under the crop in the region; increased yield, enhanced quality, reduced use of pesticides and fungicides (e.g. in varieties resistant to diseases and insect pests), savings in water (short duration of growth and drought tolerance), increased land use through early maturity to facilitate crop rotation, improved or intensified cropping systems with changed maturity or response to photoperiod. improved processing quality and value of the products (oil, starch, malt, beer and whisky), quality preference by the consumer (new flower and foliage color in ornamentals, skin and flesh color in root and tuber crops and fruit crops, aroma and glutinous nature in rice, and kernel color in wheat), increased nutritive value, highly sene and vitamins, increased oil, shelf life, reduced toxins, increased yield of essential oils, new specialty and designer crops, ease of harvest, threshing, increase in export earnings. Reduction in imports, often, induced mutations lead to more advantages than a simple desired phenotypic change.

**Table 2:** Economic impact of mutant varieties

Crop	Country	Mutant variety	Basis of value assessment	Value or area
<b>Cereals</b>				
Rice	Thailand	RD6 and RD15	Total crop value at farm gate for the period 1989–98	US\$ 16.9 billion
	China	Zhefu 802	Cumulative planted area between 1986–1994	10.6 million ha
	Japan	18 varieties	Total crop value in 1997	US\$ 937 million
	India	PNR-102 and PNR-381	Annual crop value	US\$ 1,748 million
	Australia	Amaroo	Current annual planted area	60–70% rice growing area in Australia
	Costa Rica	Camago 8	Current annual planted are	30% rice growing area in Costa Rica
	Vietnam	TNDB100 and THDB	Total planted area in 1999	220,000 ha
	Myanmar	Shwewartun	Total planted area in 1993	800,000 ha
Bread wheat	Pakistan	Jauhar 78, Soghat 90 and Kiran 95	Additional income to farmers during 1991–99	US\$ 87.1 million
Durum wheat	Italy	Creso	Additional incometofarmers during 1983–93	US\$ 1.8 billion
Barley	Uk-Scot land Numerous European countries	Golden promise Diamant and derived varieties	Crop value (1977–2001) area planted in 1972	2.86 million ha
<b>Legumes</b>				
Chickpea	Pakistan	CM 88; CM 98	Additional annual income to the growers	US\$ 9.6 million
Black gram (urdbean)	India, Maharashtra State	TAU-1	Value of increased production in season 1998–1999	US\$ 64.7 million
<b>Oil and industrial crops</b>				
Cotton	Pakistan	NIAB-78, Total value of crop from 1983– 1993	Total value of crop from 1983–1993, Additional income to growers from 1983 onwards,	US\$ 3 billion US\$ 486 million
Sunflower	USA	NuSun®	Grown area in 1994	50,000 ha
<b>Fruit trees</b>				
Japanese pear	Japan	Gold Nijisseiki	Additional annual income to growers	US\$ 30 million
Grapefruit	USA, Texas	Rio Star	Grown area (year 2000)	7,300 ha (75% of total area)

Source: B.S. Ahloowalia, M. Maluszynski\* & K. Nichterlein (2004) FAO/IAEA

### 3. Summary and Conclusion

World agriculture sustainability is threatened by increasing human populations, reduced availability of cultivated land and changing climate patterns. Plant mutation breeding is a major component in addressing these concerns in developing novel germplasm in a relatively short time. Mutation breeding is one of the breeding methods used in improving crop plants for human benefit. Different types of mutation breeding were used in the improvement of crop plants. Induced mutagenesis has played an important role by creating several mutants in different crop plants. These mutant varieties with specific character or trait such as high yield, resistance to biotic and abiotic stresses, have been grown globally bringing a significant positive economic impact and contribute to global food and nutritional security and improved livelihoods. Despite the available mutant resources, challenges still lie ahead to feed an ever-increasing population. To speed up crop production, mutant resources for different crop plants have to be established which can be used to create new mutant cultivars which are high yielding, resistant to biotic and abiotic stresses, enhanced uptake of specific metal, deeper rooting systems and modified oil, starch and protein content that can boost industrial processing.

Mutations are the heritable change to an individual's genetic makeup and are one of the driving forces of evolution complementing selection (natural and artificial) and hybridization. Mutations can create evolutionary advantages or disadvantages leading to a preferential selection or deletion of genotypes from the gene pool. Induced mutagenesis is one of the most efficient tools that has been utilized extensively to create genetic variation as well as for identification of key regulatory genes for economically important traits toward the crop improvement. Mutations are one of the fundamental forces of evolution because they fuel the variability in populations and thus enable evolutionary change. Mutations are the original

source of genetic variation and primarily create genetic diversity and mutation can have positive, neutral and negative impacts in genetic alteration of crop species. Mutation is the sudden heritable changes of genetic diversity occurred occasionally through aberration of genetic materials like DNA, RNA and protein within the cells. Mutation has a great role in increasing genetic diversity in order to feed the increasing human population. Genetic diversity is the variation occurred in genetic information, which depends on frequency and diversity of alleles among individuals within a population or a species. Mutation is the driving force for sustainable genetic diversity creation which uses in further improvement.

At present genetic variability is a narrowed using conventional breeding approach for a long period of time whereas induced mutagenesis is one of the most important approaches for broadening the genetic variation and diversity in crops to circumvent the bottleneck conditions. Induced mutagenesis can contribute to unleashing the potentials of plant genetic resources and thereby avail plant breeders the raw materials required to generate the smart crop varieties. Crop varieties generated through the exploitations of mutation breeding are significantly contributing to global food and nutritional security and improved livelihoods. Induced mutations are significant as novel mutations are being isolated for enhanced nutrition quality of crop plants. Another source of nutrition provision is from the neglected and underutilized crops, and requires more attention together with the major crops for enhancing nutrition provision to the ever-growing human population. Perhaps change of food habits would be required gradually move away from the consumption of major crops and start using underutilized crops either singly or in combination of both. Developing genetically novel germplasm with increased content of these together with other health benefit components becomes more feasible concurrent with the enhancement of breeding techniques,

genomics, molecular manipulations and genetic engineering. Induced mutagenesis is broadening the genetic variation whereas a conventional breeding approach is narrowing genetic variability for improvement for long period of time. The utilization of mutation breeding is significantly generating crop genetic diversities through improvement of crops to improve the livelihood of the communities.

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