Plant Mutagenesis and Crop Improvement

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Abstract To increase the production of food by a minimum of 70 % for the next decades is a big challenge. There is an urgent need to eradicate the hunger of an increasing human population, which is becoming disturbing because of climate change, decreasing water resources, a decline of arable land, and by the serious health and environmental hazard due to the use of agrochemicals. Increased production of quality food with low input is deemed to be a very fascinating option. On the other hand, the limitation of variations in plant crops, especially staple crops, limits the options of uncovering new alleles of genes. Hence, new variations among plant crops with new gene combinations and induced mutation is the better option thus far. Induced mutation uncovers the new combination of genes that result in a new breed with superior traits to the parents. In addition to that, cell and molecular biology methods are increasing the effectiveness and efficiency of mutation induction and detection of novel alleles of genes. Different mutagens mainly include physical and chemical mutagens and are now being applied by researchers for plant mutagenesis. This chapter reviews the methodology of mutation induction, mutagens that are being used for this purpose, and how they help us to improve the crop.

Keywords Agrochemical • Induced mutation • Physical and chemical mutagens • Mutagenesis

1 Introduction

Mainly in developing countries, the most important challenge is to attain increased productivity in a farmer's field for crop production. The Food and Agriculture Organization of the United Nations (FAO) reported that 70 % more food than today is required to feed the over nine billion human population estimated to live on Earth

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by 2050 (FAO). To meet this target, it is important to produce 37 % increased annual incremental rates (Tester and Langridge 2010). These difficulties are complex because of the finite nature of land and water resources for agriculture. In fact, in most parts of the world, counting the developing countries which are considered as serious food insecure regions (Tester and Langridge 2010; Hertel et al. 2010; Ringler et al. 2000; Nelson et al. 2009), these resources are either inactive or are decreasing because of the extra demands by economic and demographic pressures of urbanization and the need for the formation of bioenergy, livestock feeds, and many other industries (FAO). Climate changes and variations are also affecting the important land areas which then tend to become unsuitable land for crop production (FAO Beddington et al. 2011) causing major issues for food insecurity.

Therefore, it is clear that an important part of the required increase in the production of food cannot be achieved by further consumption of water resources and additional land. Because of harmful and unhealthy effects of agrochemicals to both humans and the environment, their use is not a sustainable option. Simply, more food must be produced with as much less input as possible to meet the world's demand (Mba 2013).

Cells of all living creations have similar genetic information yet they are different from each other. It is because mutation may occur in their genetic makeup resulting in the vast range of variations in them. Mutation is defined as the naturally occurring heritable change in genetic information of an individual and is considered the single most significant factor in account to the evolutionary prospectus which is passed on generation to generation for the development of new individuals, genera, and species (Mba et al. 2010). Furthermore, mutations can be used to study functions and nature of genes, and building blocks of plant development and growth, thus producing new beneficial genetic variation for the improvement of agriculture and economic crops (Adamu and Aliyu 2007).

It has been shown that variations in desired characteristics can be introduced into plants successfully through mutation and their practical value in plant improvement is well demonstrated. The major benefit of mutation is to improve one or two properties of a plant without changing the rest of its characteristics. Induced mutation has great ability and serves as an alternative technique in genetic improvement of agriculture crops (Mahandjiev et al. 2001). Induced mutation is used as a tool for major crop improvement such as in wheat, cotton, rice, barley, cowpea, and peanut. Many mutagens are used to induce mutations in crops; the mutagens include both physical and chemical mutagens (Ahloowalia and Maluszynski 2001).

2 Artificially Induced Mutation

Induced mutations offer a major increase in the production of crops (Kharkwal and Shu 2009) and the opportunity to induce desired characteristics in the plant that either have been lost during evolution or not found in nature. Mutagen treatment either breaks the chromosomes or alters the gene. Mutation occurs due to the error in DNA; this error is sometimes repaired or sometimes passed to the next generation and established in the organism genome as spontaneous mutations. Without

phenotype appearance, a mutation cannot be detected. As a result, genetic variation appears limited and breeders only have the option to induce mutation (Adamu and Aliyu 2007). For more than several decades, mutagens have been used to induce useful mutations in plants (Vasline et al. 2005). During the past 70 years, from 175 plant species, including fruits, vegetables, pulses, oilseeds, cereals, fibers, and ornamentals, more than 2543 mutant cultivars have been released in 50 countries from all over the world (Chopra 2005; Maluszynski et al. 2000).

In the 1920s, the first-ever cases of artificial induced mutation, which is genomic lesion creation above the threshold in wild-types, in drosophila, barley, and maize were reported. Induced mutation became widespread in biological sciences after these activities. In artificially induced mutation, the increase in genetic variation is done through the effectiveness of the mutagen. Many traits such as resistance to disease, culm length, yield, maturity, and lodging resistance have been subjected to mutation. It was first evaluated that the use of artificial induced mutation by mutagen to achieve genetic variation in plant crops could be useful. Mutation is induced in plants by exposing their propagules such as meristematic cells, seeds, organs, or tissues to either physical or chemical mutagens having the mutagenic characteristic (IAEA 1977). In some cases the whole plant is exposed to the mutagen. Physical mutagens exist, such as electromagnetic radiations such as UV light, gamma rays, X-rays, and particle radiations such as alpha and beta particles, and thermal and fast neutrons. In the past, irradiation was carried out by either chronic or acute ways. Farmers, on the other hand, prefer to expose plants at relatively low doses for an extended period of time of a week or sometimes even for a month; the second way is to expose plants at high doses over relatively very small periods of time such as seconds or minutes. The existing view then was that acute irradiation caused greater mutation frequencies. At present, such differences have had no apparent effect on the output of induced mutagenesis as most induction in practice is of the acute type. Chemical mutagens are mostly base analogues such as bromouracil that during DNA replication, incorporate in DNA and stand as alternates to normal bases, intercalating agents such as ethidium bromide, and alkylating agents such as commonly used ethyl methane sulfonate (EMS). The United Nations Organization (1982) and Mba et al. (2007) listed the chemical and physical mutagens that are commonly used and also their mode of action. In common, these mutagens bring changes in the DNA of the plant, changing its characteristics, traits, and appearance. Some of the examples of induced mutagenesis in plants are given in Table 1.

Table 1 Induced mutagenesis in different plants

Facilitated mutation	Plant examples
Ability of self-compatible hermaphroditism	Grapes and papaya
Failure of the hard seed coat and other germination inhibitors	Peas, wheat, and barley
Elimination of toxicity and bitterness	Lima beans, potatoes, cabbages, nuts, almonds, watermelons, eggplants
Failure of natural seed dispersal mechanism	Barley, wheat, peas
Elimination of sexual reproduction need, i.e., parthenocarpy or seedlessness	Grapes, pineapples, bananas, oranges

2.1 Mutagenesis in Plants by Physical Methods

From the discoveries of radioactivity by Becquerel in 1896, radioactive elements by Pierre and Marie Curie in 1898 and X-rays by Roentgen in 1895, man's aptitude to artificially induced mutations in plants had been started. For these inventions, the Nobel Prize in physics was awarded to Pierre and Marie Curie, Becquerel in 1903, and to Roentgen in 1901 (Mba et al. 2012a, b). Soon after these discoveries, the demonstrations of X-rays causing alteration in the genetic makeup of crops such as maize and barley (Stadler 1928, 1930, 1931, 1932) and in fruit flies (Muller 1927) were released.

Among the most commonly used physical mutagens, ionizing radiation is one of them (Mba and Shu 2012). These radiations are the part of the electromagnetic radiation (EM) spectrum having the ability to dislodge their electron from the nuclear orbits of the atom on which they act and they also have relatively high energy. The victim atom then converts into ions, thus the term ionization radiation. These ionizing components of EM consist of X-rays, cosmic, and gamma rays (γ). As far as the nonionizing radiations are concerned, such as ultraviolet (UV) light, they are able to penetrate tissue to some extent and are also used for induced mutation.

UV light is considered as a mutagen because of its capability to interlink with DNA and other biological molecules as the wavelength of UV light is absorbed by the base pairs of DNA and by amino acids in protein which are aromatic in nature. Most commonly used physical mutagens are X-rays and gamma rays (Mba and Shu 2012). Gamma rays are produced in the procedure radioisotope decay such as cesium-137 (137Cs), cobalt-60 (60Co), and to a small extent, plutonium-239 (239Pu). Gamma sources having these kinds of isotopes are typically known as gamma cell irradiators. A gamma cell is used to induce irradiation for short periods known as acute irradiation. In the exposure of the plant to irradiation for a long period of time, known as chronic irradiation, the procedure is installed in a specially designed gamma chamber or room, field, or greenhouse (Mba 2013). On the whole, the irradiations are preserved sources usually encased in stainless steel for safety measures against unintended radiations. In all ways, only an effectively trained (which depends on national statutory requirements) and certified individual operates the irradiation source. In addition to that, special precautions such as controlled access to the irradiation sources are forced on everyone to avoid any kind of mishap (Mba 2013).

The work on induced physical mutation in China and Japan in the early and mid-1990s, respectively, resulted in applications of ion beams as a useful technique in the induced mutation in plant crops (Mei et al. 1994). Ion beam irradiation is different from the above-mentioned physical mutagens and in addition to that, mass deposition, energy transfer, and charge exchange of ion beams play an important part in their mutagenecity. It is assumed that these additional properties of ion beams give additional properties and different spectra of variations to plants when exposed under it inasmuch as compared to other mutagens ion beams are generally

produced by particle accelerators, for example, cyclotrons using 14N, 56F, 7Li, 20Ne, 40Ar, or 12C as radioisotope sources (Mba 2013).

Cosmic radiations, usually known as high-energy molecules, originate from space; mainly protons have also appeared to create mutagenesis in crops (Mei et al. 1998). Mainly in China, cosmic rays in combination with microgravity and vacuum, in the general outer space environment, have been used to create useful mutations in different crops such as maize (Mei et al. 1998), tomato, cotton, sesame, sweet pepper, wheat, and rice sources (Mba 2013).

The purpose of mutagenesis is to create the highest variability in the genome with less reduction in feasibility. Of the technologies based on physical method, that is, radiation bombardment with fast neutrons and gamma rays, are now becoming most successful technologies. Among these, bombardment with fast neutrons creates large deletions, loss in chromosomes, and translocation, and irradiation with gamma rays induces small deletions and point mutations in contrast to chemical mutagens. These kinds of physical methods create damage on a large scale and badly decrease plant feasibility (Wu et al. 2005).

2.1.1 Physical Mutagenesis for the Improvement of Quality and Yield Traits

Crops propagated through vegetative propagation in combination radiation have demonstrated to be a very useful method to induce desired characteristics in plants. By inducing mutation, it is possible to induce a specific trait in the plant and make them upgraded clones (Xu et al. 2012). After soybean and palm, oilseed rapes, mainly *B. juncea*, *B. campestris* or *B. rapa* and *B. napus*, are considered the third essential supply of vegetable oil over all the world. Thus, changing fatty acid composition to improve the quality is an essential objective in improving the crop plant. But, on the other hand, they also reduce the development of the embryo and microspore embryogenesis of *Brassica napus*. Thus, choosing suitable physical mutagens is very essential for induced mutation. Analysis of the erucic acid and glucosinolate content in the mutant is done by immersing the root in 0.05 % colchicine solution for 3 h for chromosome doubling and exposure of the microspores to UV radiations. By this method, 270 double haploid line populations exhibit a high level of erucic acid and low and high levels of glucosinolate content were identified in three groups of doubled haploid lines (Barro et al. 2003).

Microspore mutagenesis use can produce mutant lines with reduced glucosinolate as compared to parents. After the UV treatment of microspores of *B. napus*, 16 mM of reduced glucosinolate content were found in contrast to 99.6 mM content in parents. The average glucosinolate in *Brassica carinata* was 80.6 mM without any physical mutagen treatment but after the exposure of its microspores under UV, the normal glucosinolate content was 37.5 mM in the mutant which is, as obvious, half of the parental (Barro et al. 2003). Barro and coworkers also demonstrated the optimization of UV treatment from the survival curve of the embryo yield after the exposure of microspores of *B. carinata*. In the doubled haploid homozygous plant

seeds, analyses of fatty acid and glucosinolate content were done with the aim of selecting lines having changed erucic acid and glucosinolate content.

As mentioned earlier, 270 double haploid line populations identified with a high level of erucic acid and low and high levels of glucosinolate content were identified in three groups of doubled haploid lines. In eight lines, the glucosinolate contents were decreased with the average of 80.6 m mol g^{-1}, however, in four lines, glucosinolate contents were enhanced up to 99.2 m mol g^{-1} per seed. The erucic acid was enhanced from 42.8 % in control lines to 49.5 % of the total fatty acid composition in some lines of additional six mutant lines. In two generations, all lines exhibited stable contents of erucic acid (Xu et al. 2012).

He et al. (2007) explained the use of physical mutagen UV for microspore isolation and embryos derived from microspores of *Brassica napus* genotypes, that is, M9, h28, h57, and h58. He also demonstrated that the treatment of UV on genotype h57 for 10 s can induce highest plant generation (55.56 %) and induction of callus (77.78 %). In cassava, cyclic somatic embryogenesis was used to induce mutation (Joseph et al. 2004). To select the appropriate experimental embryo, 50 Gy of gamma radiations is a good dose for creating mutations. It is reported that over 50 % of mutant lines were morphological varieties from the wild-type line. As a result, through this method, new cassava lines with wide morphological variations were produced.

Li et al. (2005) demonstrated the effect of gamma ray irradiation on quality, yield, and development of microtubers of *Solanum tuberosum* in vitro. Explants (propagated plantlets) of two varieties of potato, Atlanti and shepody, were exposed to five doses, that is, 0, 2, 4, 6, and 8 Gy of gamma rays, to analyze the effects of low radiation on the quality and production of microtubers in vitro.

Microtubers of both varieties were exposed to gamma rays for an extra 5 days tan control (nonirradiated). For the Atlantic cultivar, the irradiation period was extended by 10–15 days with 4, 6, 8 Gy radiations. This irradiation treatment of plantlets gave major enhancement in the number of microtubers, that is, 34.5 % and 116.7 more than wild; also it gave enhancement in fresh mass, that is, 23.2 % in the Atlantic cultivar and 77.6 % in the Shepody cultivar. Low radiation, 2–4 Gy, exposure gave enhancement in microtuber starch contents and high dose, 6–8 Gy, treatment gave increased ascorbic acid and decreased amount of sugar. Medium range 4–6 Gy exposure also caused enhanced protein contents in microtubers. explained the positive effect of gamma rays on the first generation Sudan grass. They also explained the variations in Rhodes grass with enhanced gamma rays. The exposure of Rhodes grass to gamma rays demonstrates alteration in leaf, plant height, morphology of reproductive organs, habit, and stem. Sharma et al. (1989) reported changes in many commercial traits such as green matter yield.

In rapeseed, reduction of the saturated fatty acid from normal 5.7 % to 5 %, a decrease of the linolenic acid content from 11.4 % to <8 %, and increase of oleic acid level from 47.1 % to >50 % were obtained via irradiation mutation (Ferrie 1999). The fatty acid compounds and their components have greatly affected rapeseed quality, including oleic acid, linolenic acid, unsaturated fatty acid, oil contents, and so on. Kott (1996) explained that the increase of oleic acid content from 60 %

to 85 % and reduction of linolenic acid from 10 % to 3 % were obtained by chemical mutagens. These mutagens have also proved to be the main tools to create mutations in ornamental plants and fruits. It has been reported that the in vitro shoots of Pyrus communis (pear) cultivar were exposed to 3.5 Gy gamma rays and of the selected 97 variants, only two them showed chemical behavior (Predieri and Zimmerman 2001). Microcuttings from the radiated shoot and from some nonirradiated shoots, after three subcultures, were rooted to produce plants to analyze orchard shoots. Trees with improved traits, related to their production such as consistent productivity and early bearing, were produced. In Chrysanthemum morifolium Ramat cv. Lalima, mutation in its shape and color of flower were developed by gamma rays. Ray florets were inoculated on 8.87 mM BAP and 1.07 mM NAA MS media and then exposed to 0.5 and 1 Gy gamma radiations. From 0.5 Gy, two mutants were produced and vegetatively propagated to produce true-to-type flowers. Many mutants of different fruits were produced such as increased flavor banana mutant with an early ripening in Malaysia, deep red juice and flesh in seedless grapefruit mutant in Novaria and the United States, resistant to different diseases, Japanese pear in Japan, and altered skin color of apple in Austria. On the other hand, this method yet needs improvements of clonally propagated crops including sweet potato, plantain, date palm, potato, yams, and strawberry (Ahloowalia and Maluszynski 2001).

2.1.2 Physical Mutagenesis Example

Mutational Effects of Ion Beams on Plant Crops

The ion beam effect has been analyzed not only in animals but also in plants; for example, research on *Nicotiana tabacum* and *Arabidopsis thaliana* demonstrated that in comparison to low LET radiations, ion beams are more effective in reducing the survival rate and germination rate (Tanaka et al. 1997; Hase et al. 1999). Also, analysis on glabrous (gl) loci and transparent testa (tt) showed that carbon ions (113 keV mm^{-1} produced twentyfold more mutations per dose as compared to 0.2 keV mm^{-1} electrons, hence proving the ion beam's power as a mutagen (Shikazono et al. 2003, 2005). More analysis of ion beams demonstrated that it can cause a large change in DNA such as translocation, inversion, and deletion, as well as small intergenic mutations. In comparison to electrons, ion beams usually (although not every time) create deletions with different sizes ranging from 1 bp to 230 kbp (Shikazono et al. 2005). Mutants created through ion beam can be measured as nulls or destroyed in several cases as deletion through ion beams led to total gene loss or frame-shift. It is an important difference from chemical mutagens such as EME, which usually induce point mutation by transition of AT-GC.

The major mutational ion beams effect is because of high double strand breakup (DSB) due to ions. The analysis on tobacco BY-2 protoplast as a model demonstrated that early DSB yeils were positively linked with LET and the high LET neon, carbon, and helium ions more efficient in creating DSBs in comparison to

C-rays (Yokota et al. 2007). In addition to that, it was shown that neon and carbon ions produced short fragments of DNA more consistently compared to C-rays, showing that ions can take action locally and densely on the genome to be targeted (Yokota et al. 2007).

It might be possible that ion beams induce a high rate of mutation because it is more complicated for a cell to repair DBS than single strand breakage. On the other hand, the molecular mechanism of mutation induced by an ion-mediated mechanism is yet to be explored. To resolve the issue, the breakpoints of DNA flanking sequences, generated by ion beams were analyzed and demonstrated that several analyzed sequences have 1–29 bp deletions whereas many breakpoints produced by electrons were flanked by 107 bp duplications (Shikazono et al. 2005). Based on this research, it is hypothesized that high LET ions produce DSBs as well as cause great damage to broken ends unlike electrons and these damaged ends may be removed during the natural process of repairing, hence, causing the deletion mutation (Shikazono et al. 2005).

Even though more research is necessary to clarify its accurate mode of action, ion beams can be considered an effective alternative to other mutagens such as low LET radiations and EMS that can induce high mutation and a mutational spectrum. Thus far, ion beam is used as a mutagen to a large number of plant varieties such as *Lotus japonicas*, chrysanthemums, *Arabidopsis thaliana*, carnations, and so on. It is important to analyze that this method has been thriving in the isolation of new mutant lines, doing a great job in plant breeding and genetics.

Ion Beams for Forward Genetics

Mutant isolation is only a first step in forward genetics but it is a very vital process that helps in analyzing any related gene function and giving a new vision to any physiological or developmental event. The technique of mutagenesis through ion beams has a great role in plant research, for example, by screening of M2 generation *Arabidopsis* seeds irradiated by carbon ions to produce plant lines resistant to a chemical named *p*-chlorophenoxyisobutyric acid that disturbs the auxin signaling pathway; a new mutant antiauxin resistant1-1 (aar1-1) was recognized (Rahman et al. 2006). Further analysis of this new mutant showed that the mutant displayed a satisfying response specifically to 2,4-dichlorophenoxyacetic acid (2,4D), a synthetic auxin, but did not show any response to indole-3-acetic acid (IAA), natural auxin (Rahman et al. 2006). As it has been known that both IAA and 2,4D have the same effect on the signaling pathway of auxin in spite of their stability differences, this result was very surprising. It was shown that the mutation aar1-1 is the deletion of 44 kb around eight annotated genes (Rahman et al. 2006). From them, a gene code for acidic protein, SMAP1, was revealed to be singly responsible for the phenotype of aar1-1 (Rahman et al. 2006). In addition to that, SMAP1 protein molecular analysis is important to reveal the 2,4D specific signaling pathway of auxin.

A model of legume *Lotus japonicas* has also been exposed to ion beams. Leguminous plants create a symbiotic relationship with bacteria living in soil called

rhizobia, which supply ammonia to the host plant through nitrogen fixation. As organogenesis is relatively costly, the host plants firmly control the nodule number and its growth. For this reason, legumes developed a long signaling pathway that reduces adverse extra production of nodules. A CLAVATA1-like receptor kinase gene is needed for this regulatory system and the mutations in this gene create the hypernodulation phenotype (Krusell et al. 2002; Nishimura et al. 2002; Searle et al. 2003; Elise et al. 2005). But a defined molecular mechanism has not been clarified because of the absence of other mutants of hypernodulation despite several efforts of isolating such plant lines from *N. Suganuma* and *L. japonicus* using T-DNA or EMS mutagenesis (Nishimura et al. 2002; Schauser et al. 1998; Szczyglowski et al. 1998). To avoid this problem, use of helium ions as an alternative mutagen was experimented with and a new mutant line of Lotus hyperrnodulating, clavier (klv), was released (Oka-Kira et al. 2005). Using the klv mutant for the grafting experiment demonstrated that klv is essential in shoots as compared to the root, showing that klv in combination with CLV1-like receptor kinase gene, make an extended signaling pathway that maintains the nodule number (Oka-Kira et al. 2005). This successful recognition of the klv mutant showed high mutagenic efficiency of ion beams and also demonstrated a different spectrum of mutation in comparison to T-DNA and EMS.

Ion Beam Limitations

Ion beam mutagenesis methods have been practiced with many plant varieties in the research field; as mentioned before, it has been considered a successful method to produce novel mutants. Because of its high LET properties, it effectiveness can be justified which eventually results in creating a unique spectrum of mutations, strong mutational effect, and high DSB yield as compared to other physical as well as chemical mutagens. On the other hand, ion beams have some limitations that should be considered before any experiment related to ion beams commences; for example, mutations induced by ion beams are mostly deletions that can result in total loss of gene or frame-shifting, hence, ion beams are not good for isolation of hypomorphic mutants. Furthermore, ion beam irradiation creates many other kinds of mutations such as translocation, abnormalities in chromosomes, large deletions up to 100 kb, small intergenic deletions, and inversions. Even though this large mutation by ion beams is advantageous for the induction of new mutants, the randomness of mutation patterns can effectively create problems of molecular cloning of the gene in many cases (Magori et al. 2010).

2.2 Mutagenesis in Plants by Chemical Methods

About two decades after the use of physical mutagens on crops, nitrogen mustard, and poisonous mustard gas used during World Wars 1 and 2, were shown to have the potential to cause mutations in cells (Auerbach and Robson 1946a, b, 1947)

Chemical mutagenesis is a technique to create useful mutations in plants for the improvement in their traits such as their germination ability and other related agronomic traits. Proper selection of efficient and effective mutagens is very important in any mutation program so they can produce a high frequency of desirable and useful mutations in plants. In many crop species, various chemical mutagens have been applied to obtain desirable mutations (Roychowdhury and Tah 2011a, b). On the other hand, many scientists highlighted that artificially induced mutation by sodium azide (SA), ethyl methanesulphonate (EMS), and colchicine (Col) give better access to overcome the limited variability in plants, especially carnation that produce the specific mutation without disturbing the other traits of plants (Roychowdhury and Tah 2011a, b). It might be assumed that growth abnormalities which develop in plants after chemically induced mutation are due to suppression of mitosis and cell death at different exposures to chemicals. Colchicine is a chemical known for its chromosome doubling ability, and also possesses antimicrotublar action. Sodium azide is a chemical agent responsible for creating point mutation in the DNA level and EMS is a common alkylating agent, but on the other hand, these chemicals have been shown to have great potential to produce useful mutations in the plant genome thus creating variations among a plant species. Hence, they become important techniques to increase the agronomic characteristics of crop plants (Roychowdhury and Tah 2011a, b).

Chemical mutagens are widely used because of their characteristics such as ease in handling; they do not need extra equipment and can induce maximum mutation frequency. As compared to physical mutagens, chemical mutagens alter the single base pair (bp), or single nucleotide polymorphism (SNPs). Ethyl methanesulfonate is widely used among all the chemical mutagens. During DNA replication, it creates the alkylation selectively on guanine bases resulting in the DNA polymerase replacing the cytosine residue with thymine residue opposite the *O*-6-ethyl guanine causing the random point mutation. Most transitions (70–99 %) that EMS causes are the change of GC base pairs to AT base pairs (Till et al. 2004, 2007). Mutation in coding regions of DNA can also be silent, that is, nonsense or mis-sense. In the noncoding region of DNA, mutation can cause up- or downregulation of gene transcription by altering its promoter or other regulatory regions, in the result of mutagenesis, abnormal mRNA splicing. Changed stability of RNA and alteration in protein translation may also occur.

Other chemical mutagens such as methylnitosourea (MNU) and sodium azide (Az) are also being used with the combination of Az-MNU solution. Generally, Az-MNU causes the shifting of base pairs specifically AT to GC and GC to AT. Hence, compared to EMS, the shift can go either way (18). All three chemical mutagens mentioned above can be extremely carcinogenic and must be handled with extra care. Unlike EMS, the MNU mutagen is unstable above 20 °C temperature and it is also sensitive to shock, creating difficulties to work with it. Compared to MNU and EMS which are both in liquid form in their ground state, Az is a solid dust, making it less attractive because of the additional step of dissolving the volatile substances and acute toxicity (Sikora et al. 2011).

For many years, mutagens have created a large amount of genetic variations in plants and have played a significant role in plant breeding programs all over the world. It was reported by the FAO/IAEA division in Vienna that with the help of induced mutation, 2965 crops with one or more useful traits were created during the last 40 years and released all over the world (FAO-IAEA 2011). There are many examples of crops such as barley including malting barley, cotton, grapefruit, wheat (e.g., durum wheat), rice, and sunflower that ultimately resulted in a positive economic impact (Sikora et al. 2011).

A number of scientists have reported the use of mutations to create useful characteristics and genetic variations in crop plants (Tah 2006; Khan and Goyal 2009; Mostafa 2011). In any mutagenesis program, it is important to consider the dose of chemical mutagen. In general, a higher concentration of mutagen causes greater biological damage. To increase pollen sterility, lethality, metrical traits, and germination of seed, great knowledge is required about pH value, seed soaking, chemical concentrations, and the effects of time and temperature (Khan et al. 2009). Carnation offers many chances of utilization of increasing genetic variability, recombination, and mutations in quantitatively inherited agronomic characteristics. Induced mutations are the ideal technique when it comes to improve identifiable traits. Present research has proven that genetic variability is connected with the metrical traits and germination behavior in the Dianthus crop. Hence, induced genetic variations can be effectively utilized for developing mutant strains having desirable properties (Roychowdhury and Tah 2011a, b).

2.2.1 Chemical Mutagenesis for Improving Quality and Yield Traits

EMS is a strong chemical mutagen that can alter chromosome structure. Barro et al. (2001) explained that with the treatment of EMS doubled haploid mutants with altered erucic acid were produced in *Brassica carinata*. It was done to identify the modified erucic acid level in mutant lines of *Brassica carinata*.

Nine lines were isolated from a nearly 400 doubled haploid recovered plant population that showed useful alteration in erucic acid level in the seed oil. Among these, three mutant lines exhibited below 25 %, minimum of 17 %, of erucic acid and this fatty acid concentration was more than 25 % in three lines. By microspore mutation, some very good characters related to agriculture can also be identified. In *Brassica napus*, the mutant lines with dwarf stem and long pod were attained through chemical mutagenesis using 0.2 % and 0.25 % EMS (Shi et al. 1995).

To create the new varieties of chrysanthemum (*Dendranthema grandiflora Tzvelev*), mutation through EMS in immature floral pedicels were reported by Latado et al. (2004). Pedicels of chrysanthemum cultivar Ingrid were inoculated in the 0.77 % solution of EMS for 1 h and 45 min, then washed with water for 15 min and inoculated in MS media with vitamin and salts in combination with 2 mg L^{-1} IAA, 1 mg L^{-1} BAP, and 1 g L^{-1} of hydrolyzed casein. From the population of 910 plants treated with EMS, 48 mutants were identified as having different petal color: light pink, yellow bronze, salmon pink, and pink salmon. Many of them, 89.6 % of

the total, were phenotypically uniform. Many chlorophyll mutants of *Nicotiana tabacum* anthers were identified by the treatment of EMS (Medrano et al. 1986). Stable mutants of rice anthers were also obtained by EMS treatment (Lee and Lee 2002). It has been found that the anthers treated with 0.5 % solution of EMS for 10 days after culture showed the frequencies of stable mutants, green plant regeneration, and induction of callus. EMS treated at 10 and 20 days showed stable mutant frequencies of 20.7 % and 12.0 %, respectively. Hence, it is assumed that the good timing of EMS treatment after inoculation of anther on the medium can enhance the stable mutant frequency of rice cultured anthers. Hofmann et al. (2004) reported that soybean embryonic cultures were also treated with EMS for mutation induction. It is complicated to get haploid plants in *Solanum* species through in vitro androgenesis, however; the efficiency of androgenic progeny can be enhanced by the use of EMS in *Solanum nigrum* (Kopecky and Vagera 2005).

A protocol was developed for production of physiological or agronomical mutants of two varieties of barley, Cobra and Igri, and also the production of low androgenic responding cultivar of barley by the treatment of a chemical mutagen sodium azide (NaN3) to isolate the culture of microspores in vitro (Castillo et al. 2001). The treatment of mutagen NaN3 with different concentrations was used during anther induction pretreatment right after the process of microscope isolation which makes them produce double haploid plants.

Different concentrations of mutagen EMS (i.e., 0.001, 0.01, 0.1 %) and NaN3 (i.e., 1, 10, 100 mM) were used for the isolation of microspores and embryos, at the early cotyledon stage of oilseed rape at different time intervals of 1, 5, and15 h (He et al. 2007). It was demonstrated that with a low concentration of chemical mutagen, a high yield of embryos was obtained, however, with the increase of mutagen concentrations and prolonged time period, the embryo yield gradually reduced. With the increase in EME concentrations and extended time period, the germination and survival of embryos were reduced. But, when 0.01 % EMS concentration was applied on embryos for 5 h, better results were obtained as embryo survival, high germination rate of embryos, and plant regeneration were achieved. The treatment of a low concentration of NaN3 was an encouraging effect on plant regeneration and embryogenesis in most studies of the genotype. Plant regeneration rates of genotype M9, h57, and h58 were obtained of 11.11, 15.79, and 22.22 %, respectively, when microspores were treated with NaN3 with the concentration of 10 mM for 1 h. Twenty-eight genotypes gave the highest plant regeneration rate, 19.05 %, when their embryos, developed from microspores, were treated with NaN3 with the concentration of 10 mM for 1 h. On the other hand, when NaN3 concentration was increased to 100 mM, no plant regeneration was found in all genotypes. Hence, it is indicated that use of the proper concentration of NaN3is an important step in in vitro mutagenesis.

An efficient procedure for the development of double haploids, derived from microspores of *B. juncea*, was developed with high frequency of embryo and embryogenic conversion applied to the investigation of reduced glucosinolate trait from a canola quality *B. juncea* line named Heera to a famous variety Varuna of India by backcrossing (Mukhopadhyay et al. 2007). Microspore culture showed

65–70 % production of double haploid when treated with colchicine and these microspores, when transferred to the field, exhibited a reduced mortality rate of 10 %. Freshly isolated microspores of rapeseed in the induction medium treated with colchicine for 3 days resulted in improved embryogenesis without any side effect on the embryo development (Iqbal et al. 1994; Zaki and Dickinson 1991). Isolated microspores short-term treated with colchicine enhanced the embryogenesis frequency and cell division number in *Brassica napus* (Zhang et al. 2003). After isolation of microspores, 12–15 h seems to be the optimal time for such treatments. Many approaches are being experimented with, including the other antimicrotubule compounds such as oryzalin, pronamide, trifle uraline, and amiprophose-methyle (APM) usage for doubling of chromosomes and embryogenesis during the early steps of microspore culture. The right combination of time duration for the treatment and concentration of colchicine is very important for diploidization and embryogenesis. Zhou et al. (2002a, b) reported that immediate treatment of colchicine on haploid microspores of winter and spring *Brassica napus* exhibited better diploidization and embryogenesis. Treatment of colchicine with the concentration of 500 mg L^{-1} for 15 h gave hg doubling frequency of 83–91 %. Furthermore, at this stage, very few chimeric and polyploid plants developed.

2.2.2 Chemical Mutagenesis Example

Mutational Effect of Sodium Azide on Plant Crops

Sodium azide (NaN3) is a chemical mutagen and is considered an important mutagen for crop plants. It has been shown that sodium azide is effective for physiology of plants and reduces cyanide-resistant respiration in tobacco callus (Al-Qurainy and Khan 2009). In many organisms including animals and plants, it is identified as a powerful mutagenic (Rines 1985; Owais and Kleinhofs 1988; Raicu and Mixich 1992; Grant and Salamone 1994) and it is also described in many screening assays. In different organisms, sodium azide is slightly mutagenic (Jones et al. 1980; Arenaz et al. 1989). The mutagenicity is interceded through the production of an organic metabolite of sodium azide (Owais and Kleinhofs 1988). This metabolite of azide compound first enters in the cell nucleus, makes connection with DNA, and induces point mutation in the host genome. To understand the mechanism of its mutagenicity, much research has been performed on bacteria and barley in recent years (Kleinhofs et al. 1978; Nilan and Pearson 1975). As it is a strong mutagen, it makes strong contact with plant parts and effects its growth by disturbing the plant metabolic mechanism.

Mutagenic Effects of NaN3 on Plant Parts

As mentioned above, sodium azide is a powerful mutagen and strongly inhibits the growth of parts of the plant with the increase of its concentration and treatment time period. The effect of sodium azide is explained on tomato and it is observed that it is

very effective in inducing mutation which ultimately alters root length, seedling survival, plant yield, germination percentage, seedling height, and branches per plant (Adamu and Aliyu 2007). In different crops, effectiveness of different concentrations of sodium azide treatment on root length was observed. The barley group treated with 2.5 mM of sodium azide for 3 h time period showed the lowest length of root, that is, 9.1 cm on day 14 except for the group treated with NaN3 for 3 h of time period and observed on day 7 and this exposure had a strong effect on the length of the leaf (Jia and Li 2008). The decrease in seedling survival is due to physiological disturbance and cytogenetic damage. At higher mutagenic concentrations, many factors such as changes in cell activity showed high sensitivity (Maherchandani 1975).

Effect of NaN3 on Chromosomes

To estimate mutagen potency, analysis of cytoplasm during either mitosis or meiosis is known as one of the most reliable ways. It shows a clear image of plant sensitivity for different mutagens. The nature or mutation and action mechanism induced by NaN3 is now being understood and it was started by the discovery of metabolites produced by NaN3. During mitosis, the chromosomes are damaged by NaN3 as shown in bean (Kihlman 1959), barley (Nilan and Pearson 1975; Kleinhofs et al. 1974), and leukocytes of humans (Al-Qurainy and Khan 2009). Hence, NaN3 induced abnormality in chromosomes which are either similar or a little superior to that of untreated controls. Sticky and bridge chromosomes, translocation, and lagging chromosomes are the most prominent abnormalities induced by NaN3. Inappropriate folding of chromosomes gives rise to chromosome stickiness making intermingling of chromatin fibers and chromosomes connected to each other through subchromatid bridges. With an increase in dose of NaN3, chromosome lagging also increases (Al-Qurainy and Khan 2009). The organization of spindle fibers and their movement during mitosis is dependent on ATP. Because of less availability and limited production of ATP molecules, the organization of spindle fibers in root tip cells treated with NaN3 may be disturbed, which eventually creates a disturbance in the chromosomal organization on the metaphase plate and chromosome migration toward their respective poles during anaphase (Al-Qurainy and Khan 2009). This leads to chromosomal aberrations such as sticky chromosomes, lagging chromosomes, and bridge formation as mentioned before. Hence mutagenic activities reduce the germination percentage and enhance the chromosomal abnormalities in mitotic cells of root tips (Siddiqui et al. 2007). Mutagens created changes in the structure of chromosomes and induced mutation that may be responsible for creating abnormalities in homologous chromosome pairing. Adegoke (1984) described the NaN3-induced damage in chromosomes, which results in the formation of chromosomal bridges during cell division and thus increased phenotypic changing. It also plays a major role in genetic sterility as described in rice (Mensah et al. 2005). Both colchicine and NaN3 are mutagenic agents and polyploidizing and used for a long time for creating polyploidy in plants. Ahoowalia (1967) reported the mutagenic effect of NaN3 on plant chlorophyll, yield, morphology, and sterility.

Sodium Azide Applications and Crop Improvement

For the improvement of crops, it is necessary to have genetic variability. These variations are created through natural or artificially induced mutation. This method has been used in the production of many improved cultivars and crops such as rice, soybean, vegetables, wheat, barley, ornamentals, and lupines, despite its limitation and advantages. The artificially induced mutation is a practical method to get improvement in crop genetics with the help of chemical or physical mutagens that enhance the mutation frequency as compared to natural occurrence. On the other hand, for broad use of these mutagens, high mutagenic activity is an important consideration which means that the use of a mutagen is not only dependent on its effectiveness but also on its efficiency as effectiveness of a mutagen has no implications inasmuch as chemical and physical mutagens are relatively inexpensive. However, reduced mutagen efficiency of mutagens can limit their usage (Al-Qurainy and Khan 2009). Mutagenic efficiency means the induction of a desirable trait without affecting other undesirable genetic characteristics of the plant. This is usually measured by proportion of the frequency of mutation and damage related to mutagen treatment such as chromosomal breakage, reduction in height, lethality, sterility, and so on. Mutagen usage for the improvement of crops helps in understanding the mutation induction mechanism and to quantify the frequency, also the changes of pattern in different plants by mutagens. The capability of mutagens to enter the cell of an organism for DNA interaction developed the toxic effect related to their mutagenic properties. Hence, their effects are generally because of direct interaction between the molecules of DNA and the mutagen (Al-Qurainy and Khan 2009). Some of the examples of plant crops successfully produced by the sodium azide treatment are given in Table 2.

Table 2 Plant crops successfully produced by the treatment of sodium azide

Common names of crops	Scientific names of crops	Mutated traits
Sunflower	*Helianthusannuus*	1. Reduced proanthocynidin and anthocyanin content 2. Resistant to mildew 3. Chlorophyll mutant line
Rice	*Oryza sativa* L.	1. Increased amylase content 2. Auxin resistant mutant
Groundnuts	*Arachis hypogaea* L.	Disease resistant
Oat	*Avena strigosa*	Disease resistant
Pea	*Pisum sativum*	Reduced pyridoxin
Maize	*Zea mays*	Resistant against striga (pathogen)
Sugarcane	*Saccharum officinarum*	Resistant to red rot

Mutagenic Effect of NaN3 on Root Growth and Regeneration of Somatic Embryo of Cotton

The tissue culture technique is considered an important method for plant improvement through in vitro mutagenesis, genetic engineering, and induction of somaclonal variations. Among many other techniques, plant somatic embryogenesis via callus induction is a widely used technique in various species of plants for the mutant regeneration, genetic transformation, and induction of somaclonal variations. Cotton, *Gossypium hirsutum L.*, is an essential fiber plant crop regarding the economy; in fact, 180 million humans are dependent on the production of cotton for seed oil and the textile industry (Benedict and Altman 2001). A cotton pigment produces a polyphenolic binaphthyl dialdehyde compound named gossypol and gives resistance to cotton. This compound has many characteristics such as antimicrobial, anticancer, male contraceptive, antioxidation, and anti-HIV (PICMA 1995). A proficient protocol has been developed for the increased regeneration of somatic embryo that includes oxygen supplements for the growing embryo (Shimazu and Kurata 1999), germination mediated by hemoglobin, and induced germination (Ganesan and Jayabalan 2004; Jayabalan et al. 2004).

In this analysis, in vitro mutagenesis mediated by mutagen sodium azide to develop improved frequency of cotton somatic embryogenesis was observed. In the improvement of the crop plant, mutation methods have confirmed their ability for developing useful variations. Because of in vitro difficulties in the regeneration of cotton, mutant lines of cotton regeneration were not reported (Rajasekaran et al. 1996).

To produce both genotypic and phenotypic variations, in vitro mutagenesis is an essential method. Natural variations found in regenerated plant lines are named somaclonal variations and in plantlets derived from callus, somaclonal variations are a very common mechanism. Both induce mutagenesis by mutagens and natural mutation, somaclonal variations, and result in the production of new traits without disturbing the rest of the genome. However, in contrast with natural variations, mutagenic compound induced mutations give efficient variation in plant crops. The impact and efficiency of mutagens for the improvement of crops has been reported by many scientists (Rutger 1992; Maluszynski 1990), as mentioned before. In vitro mutagenesis can help to tackle some limitations such as calculation of mutagenesis frequency of the mutated line having desired traits, the time required for regeneration of the mutated plant line, and techniques required for screening the effective mutated line (Maluszynski 1990). Chemical mutagen sodium azide is used widely due to its low cost, easy handling (Lundqvist 1992), and efficient mutagenic properties (Konzak et al. 1972).

3 Chemical Mutagenesis and TILLING

For the last decade, because of Targeting Induced Local Lesions in Genomes (TILLING) technology, the use of mutations induced by chemicals is more profound than any other method. In this technique, mutagenesis is balanced by the

chromosomal DNA isolation of all mutated lines and by the screening of the mutated population at the DNA level with the help of molecular techniques. TILLING seeds are treated with a strong mutagen, as in basic mutagenesis, which randomly induces mutation in the genome, but to achieve mutation saturation in the genome, extra care is also required. Before development of the TILLING population, most scientists started their work by developing a kill curve by the use of selected mutagens, concentration of which were plotted against survivability of the seed and the general rule of thumb mainly aiming for a survival rate of 30–80 % (Wang et al. 2010; Chawade et al. 2010). Mutagenesis then followed by seed (M1) plantation which allowed self-fertilization and development of the next seed generation (M2). Usually, one seed for each line of M1 generation was propagated to develop the M2 generation and DNA isolation was done from every single plant of the M2 generation.

Given that the high mutation number per genome and large population size, it is considered that mutated alleles of all genes are present in the population. Target crop ploidy is a main thing to consider in the analysis of the optimal TILLING population size because it is assumed that then induced mutagenesis frequency and ploidy of the targeted crop are strongly interlinked. It is demonstrated that the frequency of mutation is high in hexaploid plants such as wheat and oat without making them fertile or killing them, as compared to diploid plants such as barley and rice. That's why the TILLING population rarely needs to go above 5000 individual lines. However, the range of 10,000 lines is frequently needed in the population of diploid plants (Chawade et al. 2010; Caldwell et al. 2004).

4 Sodium Azide and Gamma-Ray Radiation Mutagenic Efficiency in Rice

Many scientists have proposed the use of two different mutagen treatments to enhance mutation induction efficiency and effectiveness. Now the question is whether these combined mutagens have any extra effect on plant mutagenesis regarding mutation frequency. Lately, some mutagen combinations were tested on rice; that is, gamma rays were combined with different chemical mutagens such as MMU, HA, and NMU (Rao and Rao 1983), EMS (Rao 1977), DES and EMS (Kaul and Bhan 1977), and SA (Reddi and Rao 1988).

At the Centro de Energia Nuclear na Agricultura, in Piracicaba, São Paulo, 500 dormant and dried Brazilian rice variety IAC-1246 seeds were exposed to gamma rays of 10, 15, 20, and 30 Kr of Co60 source. In an experiment chlorophyll mutant lines of rice were created by the combination of sodium azide and gamma rays and with sole treatment of each mutagen as well. The azide treatment gave the highest percentage results of chlorophyll mutagenesis in rice, followed by combined treatment of sodium azide and gamma rays and the least percentage was given by gamma ray treatment. On the other hand, M2 plants mutant frequency was more efficient in combined treatment of 5 mM sodium azide and 15 Kr gamma rays than any of the

solo mutagen treatments. In general, the mutagenesis frequency is more effective in sodium azide as compared to gamma rays (Ando and Montalván 2001).

5 Past Achievements in Induced Mutagenesis

The main strategy in mutation has been to improve the adapted varieties of plant by changing their one or two important characteristics. These characteristics include seed shattering, plant maturity, and height and disease resistance, which increased the quality and yields with traits such as malting quality, oil content and profile, quality, and size of starch granules. For example, short height mutation in wheat, maize, barley, and rice has significantly contributed to enhance grain yield. This allowed the use of nitrogen application in high doses. With the use of chemical and physical mutagens, various such types were being produced, for example, Calrose 76 (the semi-dwarf rice mutant) released in California has the property of stiff and short straw and has a main role in contributing to rice production in the United States, as did as the short height rice mutant named Basmati 370 in Pakistan. In India, many rice mutants via gamma radiation were released with maximum yielding cultivars under the series PNR; some are in early maturation and had small height (Chakrabarti 1995). Zhefu 802, considered an outstanding mutant of rice, was grown in China over more than 10.6 million ha for 10 years. In 1977, an aromatic indica variety of rice named RD6 was produced in Thailand through gamma radiation. This variety is still grown in Thailand even several years after its release. During the wet season of 1994–1995, this variety was planted on about 2.4 million ha (15.2 rai) which covered the 26.3 % area under rice during the season. In 1978, another mutant called RD15 was released which was planted over 0.2 million ha, equal to 3.2 % area under rice (Anonymous 1995). In *Japonica* rice, a thermosensitive genic male-sterile mutant, controlled by a recessive gene, has helped mainly to develop methods for production of hybrid rice varieties. In China, similar mutants were introduced by using gamma rays in indica rice named 26 Zhaizao (Ahloowalia and Maluszynski 2001).

Several mutants are released as cultivars; many others have been used as parents for the production of new cultivars. In Europe, the production of short height and high-yielding mutants of barley named Golden Promise and Diamant have had a major effect on the brewing industry. These mutants were then used as parents for many new barley cultivars in 1987, and had a main effect on supporting the development of the textile industry in Pakistan. This cultivar has the properties of determinate growth rate, heat tolerance, and bollworm attack resistance because of its early maturity, which made it a perfect cultivar for cotton and wheat rotation. In India, in the early years of the Green Revolution, the production of a mutant of Sonora named Sharbati Sonora showed great acceptance by customers because of its better grain color. Many high-yielding, early-maturing varieties of peanut called Yueyou series were produced in China, which were produced from crosses with mutants developed by radiation. A mutant variety of peanut TG-26 was recently developed at the Bhabha Atomic Research Center, Bombay, India.

At the farm level, India yielded 9.4 tons/h nuts. Based on induced mutation (Green 1986; Dribnenki et al. 1996), the production of a cultivar with good oil quality named linola, has the most recent and important role in changing oil quality as it had been done before in high oleic acid in sunflower and in canola from rapeseed. At present, some mutants that have reduced glycoalkaloid content in potato tuber (Love et al. 1996) have been reported. But the technology still needs more improvement for the propagation of crops such as yams, strawberry, plantain, date palm, and sweet potato.

Recently, mutants have been reported for reduced glycoalkaloid content in potato tubers. On the other hand, the technology has yet to be tested for the development of clonally propagated crops such as sweet potato, date palm, yams, plantain, and strawberry (Ahloowalia and Maluszynski 2001).

6 Use of Induced Mutations in Basic Research

6.1 Developmental Mutants

In *Arabidopsis*, various mutants have been used to observe genes that show response to plant growth hormones, that is, auxins, cytokinins, gibberellin, abscisic acid, and ethylene in the development of floral, formation of fruits, plant growth, senescence, and fruit ripening. The mutants make identification, isolation, and cloning of the genes possible, which helps to produce crops with increased tolerance to stresses, reduced agronomic inputs, improved yield, and longer shelf life (Ahloowalia and Maluszynski 2001).

There are induced mutations in many crops such as maize, pea, tobacco, barley, and *Arabidopsis thaliana* for the isolation and identification of genes that control the development of plants, especially floral parts formation, fruit ripening, formation of fruit and seeds, and the onset of flowering. These mutants engage growth hormones, phytohormones, including auxins, cytokinins, gibberellin, abscisic acid, ethylene, and brassinosteroids. The study of regulation of phenotype at the molecular level and biosynthesis level in plants had been a slow procedure (Ahloowalia and Maluszynski 2001).

The dissection of loci is now possible due to the ease with which many mutants can be identified and isolated among the *Arabidopsis* mutagenized population. In various cases, to study plants with different traits at the morphological as well as biochemical level, these genes have been isolated and cloned. In addition to that, in *Arabidopsis*, mutagenesis based on T-DNA insertion increased our knowledge of plant biochemistry, physiology, and development. Without the process of induced mutation, mutant production, and mutagenesis basis, this progress would not have been possible. The study of a mutant, named super root (sur1) which produces extra free auxins showed the genetics basis of auxin synthesis regulation. Another mutant, fass, reduces elongation of cells in the basal and apical axis, and demonstrated the usage of locus in auxin homeostasis and auxin conjugation (Ahloowalia and Maluszynski 2001).

There are various mutants that are effective for auxin transduction (axr1, axr4), auxin uptake, auxin inhibition, and auxin transport (aux1, pid, mp, lop1), and many others that are being used to analyze auxin metabolism (Leyser 1997). In the same way, many mutants with changed response to cytokinins are now accessible that help to create a better understanding of the nature of cytokinin action. Such mutants include photomorphogenesis mutants (det1, cop), cell division mutants, increased cytokinin levels (amp1), and cytokinin resistant mutants. With the decrease/increase in the specific transcripts, gene expression of plants can be effectively changed in response to cytokinins. Such kinds of genes are usually regulated by extra change such as auxins and light. In *Arabidopsis thaliana*, many mutants of cytokinin and related to its metabolism such as zea, cry1, ckr1, stp1, and ein2 have been isolated. They are cytokinin resistance and have explained that genes for cytokinin regulation may be involved in many biological processes such as photosynthesis, disease resistance, chloroplast development, and nutrient metabolism (Schmülling et al. 1997). Understanding main processes is important to understand plant crops with enhanced growth rate, yield, resistance to disease, and better nutrient uptake. In Japan, *Gibberellafujikuroiis* used to infect elongated rice seedlings resulted in the isolation of gibberellic acids in the form of crystalline (Yabuta and Sumiki 1938). In comparison to other cytokinins and auxin, mutants with changed elongation of shoot in maize and pea were used to analyze gibberellins early in 1955 and 1956. The mutation restored the wild-type phenotypes of dwarf mutants of maize and pea by the application of GA3. From then on, many mutants involved in enzymes that catalyze GA biosynthesis pathways (ls-1 and lh-2 in pea, an1 and d3 in maize, dx in rice, and gal-3 in *Arabidopsis*) and directly in GA synthesis, were isolated in maize, wheat, *Arabidopsis*, rice, and pea. A dwarf mutant of barley deficient in GA showed reduced amylase activity. Some other mutants such as spy and gai in *Arabidopsis* are GA responsive. Some mutants short in height such as D8 in maize and Rht3 in wheat are deficient in GA and do not respond when GA3 is applied (Ross et al. 1997). Improvement of several cereals such as rice, barley, wheat, and sorghum, and dwarf mutant whether induced or natural, have played an important role in the development of high fertilizer responsive and lodging resistance varieties.

The genetic study of signal transduction of abscisic acid has been based on several mutants deficient in ABA such as aba2 in *Nicotiana plumbaginifolia* and aba1 in *Arabidopsis* that are orthologous as revealed by mapping and transposon tagging isolation. Other mutants that give altered response to application of ABA such as abi1, abi2, abi3, and abi4 showed a marked decrease in the germination of seed (Merlot and Giraudat 1997). Such types of genes are highly useful for cereal varieties that sprout in situ in the period during seed maturation. A mutant etr1 of *Arabidopsis* that gives synthesis of ethylene has a main role in enhancing the shelf life of fruits, delayed senescence, and extended flower life demonstrated by its transfer to petunia and tomato (Wilkinson et al. 1997). Many such types of mutant such as the Nr mutant of tomato and ain, einif *Arabidopsis* have very limited response to ethylene. These mutants also have an important role in the trade of fruits such as mango, banana, pineapple, cut flowers, and papaya which spoil after ripening. Mutants that develop defective flowers known as homeotic mutants have been

isolated in petunia, *Arabidopsis*, *Lycopersicon*, and*Antirrhinum*. Three groups of gene A, B, and C, single or in combination, control the development of organs in the four whorls of dicot flowers. These are the mutations in homeotic gene, GREENPETALS in petunia, FLORICULA, PISTILLATA, AGAMOUS, and SQUAMOSA in tomato (designated TAG1 that alter structures of flowers such as sepals, petals, anthers), GLOBOSA, DEFICIENS A, APETLA3, PLENA, and AGAMOUS in *Antirrhinum* lechomeotic mutant of leafy cotyledons, developed via insertion mutagenesis in *Arabidopsis*, are defective for embryo maturation that remain green (Meinke 1992).

The fis mutants that controlled the development of seed without fertilization, have an important role in understanding apomixes (Chaudhury et al. 1997). The mutant isolation that determines development of fruits, seeds, and flowers, has an important role in our understanding of the general patterns of plant development. The quality and yield of crop plants are determined by the development patterns in plant crops. The options of changing them will open new ways in plant genetics. The recent study on the maize mutant INDETERMINATE (ID1) verifies the signal translocation from the shoot apical meristem, where it develops flowering, to the leaves. This might be considered as a first step to the subtle florigen, concerned in the photoperiodic response of plant flowering (Ahloowalia and Maluszynski 2001).

6.2 Mutants for Changing Starch Quality

Starch is an important carbohydrate that is stored in tubers and seed amyloplasts. Most starch is produced in a few crops such as maize, oats, barley, potato, banana, rice, sorghum, cassava, sweet potato, plantain, and wheat. It can be divided into two macromolecules, amylopectin and amylose. Amylose is an important starch in linear shape formed of anhydroglucose units linked glycosidic linkage. On the other hand, amylopectin has many millions molecular weight and is in branched polymer developed by anhydroglucose units yet with 2–4 % branched form (Ahloowalia and Maluszynski 2001).

In most plants, starches are formed of about 70 % amylopectin and 30 % amylase. Mutation in biosynthesis of starch can change the concentration of both components that eventually will change the physical as well as chemical characteristics of starch granules and has been demonstrated in wheat, pea, rice, and maize. In maize, a large number of mutants are identified (Nelson and Pan 1995; Creech 1965). Among these mutants are the sugary (susu) in maize (Hannah et al. 1993) that has waxy (wx loci) and debranching characteristics.

In pea, several mutants have been induced by chemical mutagens (Blixt 1972). Six loci have been identified in pea that change the composition and use of starch. In one of them, the regosus loci (r), the dry seeds are wrinkled as explained by Gregor Mendel (Mendel 1865). Mendel analyzed the mutations derived because of a transposonlike addition in the gene (Bhattacharyya et al. 1990). Chemical mutagenesis produces single base pair changes in all alleles (MacLeod 1994). A main

gene named Hardness (ha), located on the short arm of chromosome D of wheat controlled the texture gene (soft or hard). On the 5A and 5B chromosomes of hexaploid wheat, alleles for the hardness gene are present but not expressed. A 15 kDa marker protein for the softness of grain named Friabilin is constituted of two proteins, puroindoline a and b (pinA and pinB). This protein (soft starch) is present on the water-washed starch surface in higher concentration whereas hard wheat starch is in a small concentration. It is missing in the starch of durum wheat. Recent studies showed that the softness and hardness of wheat grain (*Triticum aestivum* L. em Thell) is connected to point mutation of glycine to serine in pinB or null mutation in pinA that follows the absence of pinA protein. These mutations demonstrate the hardness of grain. The complete connection between the hard texture of grain and mutation in pinB among 5D chromosomal substitution lines showed that pinB is involved in control of the texture of grain. It appears that mutation in either component of pinA, pinB, or friabilin can alter the hardness of grain (Giroux and Morris 1998).

7 Conclusion and Future Prospective

The currently available varieties of most staple crops do not fit into the vision of a highly effective but low-input system of crop production. This means a novel portfolio of cultivars of plant crops will need to be developed. By inducing mutation in crops, the potential of scientists of understanding and developing fundamental genic control that changes the expression of agronomic and crop characteristics to greatly improved. Consequently, induced mutagenesis is now an approach widely used in functional genomics as it greatly helps in gene identification and the explanation of their functions. Interestingly, functional genomics output named elucidated genes when used as molecular genetic markers, increasing the plant efficiency. Scientists are now applying other methods in addition to chemical and physical mutagenesis, such as combination of both chemical and physical mutagens and combination TILLING with mutagenesis.

This chapter demonstrates that techniques such as physical and chemical mutagenesis can be used to produce plant genotypes with the desired properties. Researchers have the goal to generate the tools that are the most suitable and effective for producing the desired genotypes (Bregitzer et al. 2002). On the other hand, it is still hard to guess if transgenic food could become the “norm” for ordinary consumers in the next few years. Somaclonal variations may be the more reliable mechanism for the early detection of desirable traits. Thus, induced mutagenesis techniques that gained great interest about the middle of the twentieth century are now worthy of further analysis and improvement by the use of different methods for the improvement of plants in the twenty-first century (Smith 2008).

Plant selection with the required properties is more significant compared to the methods used for mutation or production of variation. Consequently, the use of molecular probes gives a great chance in this regard. Molecular techniques with

probes will become more significant in mutagenesis techniques, especially for changing superiority traits such as protein, starch, oil, and others in crop plants for industrial processing.

In vitro culture techniques decreased the quantity of cultural material up to a milligram; only small quantities of tissues and calli are used for mutation, and may be decreased to micrograms in the coming era when daily techniques are generated for these methods. Nowadays, the number of vegetatively propagated plants including sugarcane and banana, produced via in vitro mutagenesis is very low. However, many seeds propagated like maize, barley, soybean, rice, wheat, and rapeseed, among others are generated through cell-suspension culture. Although there are some problems, such as cells in suspension culture turn into clumps, it is expected that the irradiation dose needed for cell suspension culture for induced mutation would be lower than for callus culture. Hence, we should look forward to the advancement of daily techniques for seed and also for vegetatively propagated crops. Moreover, generation of methods of in vitro cell-selection for disease and toxin resistance can be used in culture media.

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