

Review

Induced Mutations Unleash the Potentials of Plant Genetic Resources for Food and Agriculture

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Abstract: The options for increasing food production by at least 70% over the next four decades so as to keep pace with a rapidly increasing human population are bedeviled by erratic climatic conditions, depleted arable lands, dwindling water resources and by the significant environmental and health costs for increasing the use of agrochemicals. Enhanced productivities through “smart” crop varieties that yield more with fewer inputs is a viable option. However, the genetic similarities amongst crop varieties—which render entire cropping systems vulnerable to the same stresses—coupled with unvarying parental materials limit the possibilities for uncovering novel alleles of genes and, hence, assembling new gene combinations to break yield plateaux and enhance resilience. Induced mutation unmask novel alleles that are harnessed to breed superior crop varieties. The historical antecedents, theoretical and practical considerations, and the successes of induced mutations in crop improvement are reviewed along with how induced mutagenesis underpins plant functional genomics. The roles of cell and molecular biology techniques in enhancing the efficiencies for the induction, detection and deployment of mutation events are also reviewed. Also, the integration of phenomics into induced mutagenesis and the use of pre-breeding for facilitating the incorporation of mutants into crop improvement are advocated.

Keywords: induced mutations; mutagens; mutant; crop improvement; genomics; cell biology; TILLING; phenomics; pre-breeding

1. Addressing the Challenge of Feeding the 21st Century World

A most critical challenge to crop production is the attainment of enhanced productivities in farmer's fields, especially in developing countries. The Food and Agriculture Organization of the United Nations (FAO) estimated that 70% more food is needed to feed adequately the over nine billion people expected to inhabit planet earth by 2050 [1]. The task of meeting this target, which requires a 37% increase over the historical annual incremental rates [2], is clearly as daunting as the scope of the envisaged increases is unprecedented. The difficulties are compounded by the finite nature of arable lands and agricultural water resources. Indeed, in most parts of the world—including the critically food insecure regions of the developing world [3–6], these resources are either stagnant or are dwindling on accounts of additional demands for them by the demographic and economic pressures of urbanization and the competing needs for producing livestock feeds, bioenergy production and several other industries [1]. The effects of climate change and variations are also rendering significant areas of arable lands unsuitable for crop production [7–9]; this is a major driver for food insecurity.

1.1. Enhanced Productivities on Farmers' Fields Are Critical

It is obvious therefore that a significant portion of the required increases in food production cannot be attained by the further deployment of additional land and water resources. The increased use of agrochemicals is also not a sustainable option on account of its deleterious impacts on health and the environment. Simply, more food must be produced with fewer inputs. The admixture of complementary solutions being adduced for feeding the world's teeming population with fewer agricultural inputs and with minimal ecological footprints constitute the "greener", ecosystem-based and knowledge-intensive paradigm that is commonly referred to as sustainable crop production intensification [10,11].

FAO [11] recommended that, for realizing the imperative of the low-input agriculture being proffered for the 21st Century, farmers require a suite of improved crop varieties that are genetically diverse, climate change resilient, input use-efficient, high yielding, have enhanced nutritional and other quality attributes and have been bred for adaptation to a range of agroecosystems and farming practices. But, the envisaged genetically diverse portfolio of suitable crop varieties are neither available to farmers [2,12,13] nor do the current breeding strategies hold promise for delivering them [14]. The extremely narrow genetic base of the available varieties of crops and the parental lines for breeding new ones nullify efforts to enhance productivities in farmers' fields, increase vulnerabilities and thereby imperil food security.

1.2. The Unmasking of New Alleles Increases Chances for Success

Clearly, the devising and adoption of winning strategies for addressing these dire scenarios necessitate a profound re-evaluation of all aspects of the crop production value chain including the suitability of the crop varieties that farmers grow. There are several mechanisms for facilitating the enhanced harnessing of the inherent potentials encoded into the genetic blueprints of crops so as to make available wider sources of heritable variations to crop improvement. Mba *et al.* [14] have suggested that, in addition to pre-breeding strategies that involve the increased incorporation of traits

from non-adapted genetic resources including landraces and crop wild relatives in crop improvement, putative parental materials can also be induced to mutate as means for unleashing new alleles of genes that control the traits desired for the “smart” crop varieties of the 21st Century. We review the exploitations of spontaneous and induced mutations in crop husbandry and genetic improvement. The advances made in the uses of contemporary scientific and technological methodologies to enhance the efficiency of the induction, detection and deployment of induced mutations are also explored. We also provide perspectives for facilitating the integration of induced mutations in plant breeding programs.

2. Mutation

Mutation is the heritable change to the genetic material; individuals that manifest modified characteristics on account of heritable changes are known as mutants. In nature, the combinations of the errors that occur during the replication of deoxyribonucleic acid (DNA) and damages to this hereditary material on account of an individual’s exposure to sunlight and ultra-violet (UV) radiation and diseases lead to heritable changes to the genetic blueprint. Other types of mutations include duplications whereby whole parts of the genome are doubled and translocations whereby a part of a chromosome is transferred to a non-homologous chromosome; these occur as a result of errors during meiotic cell division. Their effects depend on whether or not, and how, genes are affected in such mutations. The accumulations of such aberrations have been the primer for evolution and speciation.

Stretches of DNA make up the basic unit of heredity, the gene. The subtle changes to DNA sequences are the most useful for crop improvement as the more gross alterations (at the chromosomal and cellular levels) invariably confer some levels of unfitness and even lethality. One way for classifying mutations at the DNA sequence level is to categorize the mutation on the basis of how the modification affects the gene’s ability to synthesize the protein that it is responsible for. In general, the mutations that are important in crop improvement usually involve single bases and may or may not affect protein synthesis.

2.1. Common Types of Mutations Relevant to Crop Improvement

The common types of mutations in DNA sequences include:

2.1.1. Single Base Substitutions or Point Mutations

One base is replaced by another could be either transitions or transversions. In a transition, one purine (A or G) or pyrimidine (C or T) is replaced by the other. In situations where a purine is replaced by a pyrimidine or vice-versa, this is known as transversion. Point mutations include:

- Samesense, silent, or wild-type mutation occurs when the substitution does not affect the amino acid that is encoded by a codon; this is because many amino acids are encoded by different codons. The mutation in this case can only be detected through sequencing (or some other appropriate molecular assays) as there is no detectable phenotypic change.
- Missense mutation refers to the situation where the new nucleotide modifies the codon such that an altered amino acid is encoded for in the protein. This results in detectable phenotypic changes.

- Nonsense mutation describes the substitution that results in a codon that instead of encoding an amino acid is modified into one of the stop codons. This results in the premature termination (or truncation) of the translation of codons to amino acid sequences in the proteins leading to a protein with shortened polypeptides that might be inactive. Severely truncated proteins, *i.e.*, those with the truncation sites early in the gene sequence are inactive.

2.1.2. Insertions and Deletions (Indels)

Mutations involving situations where extra base pairs, ranging from one to thousands, are inserted into (insertion) or deleted from (deletion) the sequence of a gene are referred to as indels. Indels result in shifts in the reading frame of the gene with the rearrangement of the nucleotides into a completely new set of codons. This has grave consequences for protein synthesis as the amino acid sequences are manifestly different from the wild-type. Indels of one or two base pairs (or multiples) result in frameshifts while those of three base pairs (or multiples) are more innocuous as the reading frame tends to be preserved, *i.e.*, the original triplet codons are not disturbed.

2.2. Spontaneous Mutations

Spontaneous mutants are aberrant types that are found in nature and for which no deliberate intervention by man has engendered the novel phenotypes. The domestication of off-type plants, *i.e.*, spontaneous mutants that manifested traits suitable for crop husbandry, about 10 millennia ago contributed immensely to the origins of agriculture and hence, the current relatively sedentary lifestyles and permanent human settlements.

The loss of certain “wild” characteristics in crops through spontaneous mutations was the principal driver in the founding of agriculture in the Fertile Crescent of West Asia and independently in northern and southern China, Africa’s Sahel, New Guinea, the Andes and several regions of the Americas. Notable examples of spontaneous crop mutants whose heritable spontaneously altered characteristics led to domestication and ease of cultivation and hence ready availability for human consumption include peas, wheat and barley. The accumulation of spontaneous mutations that abolished pod or head shattering (natural seed dispersal mechanisms) and the thinning of seed coats resulting in reductions in lengths of seed dormancy periods made these plants suitable for cultivation at will. Another important example of a heritable permanent change that, in distinguishing mutants from their wild-types, made them suitable for human consumption is the loss of bitterness in almonds, lima beans, watermelons, potatoes, egg-plants, cabbages and several types of nuts. Other spontaneous mutations that have become advantageous include parthenocarpy in bananas and grapes. Table 1 provides a list of some spontaneous mutations that have facilitated the processes of crop domestication over time and hence settled agriculture.

Table 1. Spontaneous mutations and domestication of crops.

Mutation that facilitated domestication	Examples of plant
Abolishment of bitterness and toxicity	almonds, lima beans, watermelons, potatoes, egg-plants, cabbages nuts
Abolishment of the need for sexual reproduction (seedlessness or parthenocarpy)	bananas, grapes, oranges, and pineapples
Loss of natural seed dispersal mechanism—shattering of pods and heads	peas, wheat, barley
Loss of the hard seed coat and other germination inhibitors (dormancy)	wheat, barley, peas
Facility for self-compatible hermaphroditism	grapes, papaya, <i>etc.</i>

More recently, the green revolution, a term used to describe the huge increases in grain yields from the 1960s, was made possible through the introgression into wheat and rice of spontaneous mutant alleles of genes that, in conferring reduced plant height (and stiffer lodging-resistant straws), are known as dwarfing genes. Unprecedented stable high yields were achieved for dwarf wheat varieties and marked an epochal defining moment in international agricultural research and development. Mutation induction in crops therefore represents man's successful attempt at reproducing and scaling up the frequency of a naturally occurring phenomenon so that it can be exploited advantageously.

2.3. Induced Mutations

Agents that are used to induce hereditary changes are broadly divided into physical and chemical mutagens. Crops are induced to mutate through the exposure of their propagules to physical and chemical mutagenic agents. For seed propagated crops, botanical seeds are treated with the mutagens while for vegetatively propagated plants, other plant parts used for propagation such as stem cuttings, twigs, buds and tubers are exposed to the mutagenic agent. More recently, the induction of mutations in vegetatively propagated plants is becoming more efficient as scientists take advantage of totipotency, *i.e.*, the inherent ability of individual plant cells to regenerate into whole plants, to use single cells and other forms of *in vitro* cultured plant tissues as starting materials for the induction of mutations.

2.3.1. Physical Mutagens

Man's ability to deliberately induce mutations in plants derives directly from the discoveries of X-rays by Roentgen in 1895; radioactivity by Becquerel in 1896; and radioactive elements by Marie and Pierre Curie in 1898. For these achievements, the Nobel Prize for Physics was awarded to Roentgen in 1901 and to Becquerel, Marie and Pierre Curie in 1903 [15]. Deriving directly from these discoveries were the seminal demonstrations soon afterwards that radiation (X-rays) caused alterations to the genetic make-up of fruit flies [16] and in the crop plants—maize and barley [17–21]—

respectively. These proofs of concept proved to be the watershed moments in induced mutagenesis as they provided the impetus for the subsequent widespread adoption of this mimicry of nature in crop improvement and more recently as a strategy to discover genes and elucidate their functions.

Ionizing radiations constitute the most commonly used physical mutagens [22,23]. These are parts of the electromagnetic (EM) spectrum that, on account of their relatively high energy levels, 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 (γ) and X-rays. Ultra violet (UV) light, though non-ionizing, is capable of some level of tissue penetrability and has been used in inducing mutations as well. The mutagenicity of UV derives from its ability to react with DNA and other biological molecules as its wavelengths are preferentially absorbed by bases in DNA molecules and by the aromatic amino acids of proteins.

In addition to the ionizing radiations, the other commonly used physical mutagens are the high energy ionizing particles, alpha (α) and beta (β) particles and neutrons. The properties of physical agents that are used in inducing crops to mutate are summarized in Table 2. The mutagenicity of these agents derives from a combination of their ability to produce dimmers and reactive ions which in turn cause damage to living organisms; the damage caused ranges from aberrations at the DNA level (breaking the chemical bonds in DNA molecules, deleting or adding nucleotides and by substituting one nucleotide for the other) to gross chromosomal breakages and rearrangements.

Table 2. Some commonly used physical mutagens and their properties.

Mutagen	Typical Frequency (s^{-1})	Typical Energy (kJ/mol)	Typical Photon Energy (eV)
Particles			
α -particles		4.1×10^8	
β -particles		1.5×10^7	
Electromagnetic radiation			
cosmic rays	$6 \times 10^{21} s^{-1}$	2.4×10^9	
γ -rays	$3 \times 10^2 s^{-1}$	1.2×10^8	1 MeV
x-rays	$3 \times 10^{17} s^{-1}$	1.2×10^5	100 keV
ultraviolet	$3 \times 10^{15} s^{-1}$	1200	4 eV

Gamma and X-rays are the most commonly used physical mutagens [22,23]. Gamma rays are emitted in the process of the decay of the radioisotopes cobalt-60 (^{60}Co), cesium-137 (^{137}Cs) and to a less extent, plutonium-239 (^{239}Pu). Gamma sources containing one of these radioisotopes are typically installed as gammacell irradiators. A gammacell is used mostly for acute irradiation (i.e. for short periods). For chronic irradiations, i.e. the exposure of plants to irradiation for extended periods of times, irradiators are installed in specially designed gamma rooms (or chambers), greenhouses or fields. In general, the irradiators are sealed sources which are normally encapsulated within a stainless steel casing as a safety measure against unintended irradiation. In all cases, only adequately trained and, depending on national statutory requirements, certified personnel operate the sources. Also, special precautions, including strictly controlled access to the facility, are imposed in order to prevent accidents.

The implantation of ion beams is rapidly gaining currency in the induction of mutations in crops since the pioneering work in China and Japan in the early and mid-1990s, respectively [24,25]. Ion beams differ from aforementioned physical mutagens in that in addition to energy transfer, mass deposition and charge exchange play vital roles in their mutagenicity. It is postulated that these additional properties predispose the treated plant materials to the production of spectra of variations that are distinct from those produced through exposure to other mutagens. Ion beams are usually generated by particle accelerators, e.g., cyclotrons using ^{20}Ne , ^{14}N , ^{12}C , ^7Li , ^{40}Ar , or ^{56}Fe as radioisotope sources.

Cosmic radiation, the collective term for high energy particles that originate from outer space (mostly protons), has also been demonstrated to be mutagenic to crops [26–28]. With China playing the leading role, strong cosmic radiation in conjunction with the super vacuum and microgravity that characterize the outer space environment, have been used to induce useful mutations in rice, wheat, cotton, sweet pepper, tomato and sesame [29] and in maize [27]. Induced mutations, exploitable in crop breeding, have thus been achieved by transporting plant propagules, mostly botanical seeds, in recoverable space orbiting satellites. It is plausible, though, to infer that the highly specialised nature, expense and start up infrastructure make the use of cosmic radiation the most improbable choice for most scientists.

2.3.2. Chemical Mutagens

It would be about two decades after the demonstrations of the mutagenicity of physical agents that nitrogen mustard (component of poisonous mustard gas used in World Wars I and II) would be shown to cause mutations in cells [30–38]. This would pave the way for the identification of several other chemical mutagens; these include base analogues, alkylating and intercalating agents and chemicals that modify DNA structure. Their effects on DNA molecules manifest in deamination, the induction of transitions and insertions, the stoppage of transcription and replication and even strand breaks. The properties of the chemical agents commonly used for inducing mutations in crops are shown in Table 3.

Table 3. Some commonly used chemical mutagens and their modes of action.

Chemical mutagenic agent	Mode of action
Base analogues e.g., 5-bromouracil (BU), 5-bromodeoxyuridine, 2-aminopurine (2AP)	Incorporates into DNA in place of the normal bases during DNA replication thereby causing transitions (purine to purine or pyrimidine to pyrimidine); and tautomerization (existing in two forms between which they interconvert e.g., guanine can exist in keto or enol forms).
Nitrous acid	Acts through deamination, the replacement of cytosine by uracil which can pair with adenine and thus from subsequent cycles of replication lead to transitions.

Table 3. Cont.

Chemical mutagenic agent	Mode of action
Alkylating agents such as: sulfonates e.g., ethylmethane sulfonate (EMS), diethyl sulfonate (DES); Sulphur mustards e.g., ethyl-2-chloroethyl sulphide; Nitrogen mustards e.g., 2-chloroethyl-dimethyl amine; and Epoxides e.g., ethylene oxide Others are ethyleneimine, hydroxylamine (NH ₂ OH), <i>N</i> -methyl- <i>N'</i> -nitro- <i>N</i> -nitrosoguanidine (MNNG), sodium azide and diazomethane.	They react with bases and add methyl or ethyl groups and, depending on the affected atom, the alkylated base may then degrade to yield a baseless site, which is mutagenic and recombinogenic, or mispair to result in mutations upon DNA replication.
Intercalating agents such as acridine orange, proflavin, ethidium bromide	They insert between bases of DNA thereby causing a “stretching” of the DNA duplex and the DNA polymerase in turn recognizes this stretch as an additional base and inserts an extra base opposite this stretched (intercalated) molecule. This results in frameshifts <i>i.e.</i> , an alteration of the reading frame since codons are groups of three nucleotides.
Miscellaneous group of agents; Large molecules referred to as “bulky” lesions (e.g., <i>N</i> -acetoxy- <i>N</i> -2-acetyl-aminofluorine—NAAAF).	They bind to bases in DNA and cause them to be noncoding thereby preventing transcription and DNA replication; They cause intra- and inter-strand crosslinks (e.g., Psoralens); They also cause DNA strand breaks (e.g., peroxides).

3. An Induced Mutagenesis Program

The generalized scheme for induced crop mutagenesis—as a crop improvement strategy—is straightforward and involves the sequential steps of the exposure of plant propagules to pre-determined doses of a mutagen, the identification of stable mutants amongst the progeny and the incorporation of the desirable mutants into breeding programs or the use of mutant stocks for the identification of genes and the elucidation of their functions. To be successful, the researcher would have to make informed decisions that range from the choice of mutagen through the doses to be administered to the handling of the putative mutants.

3.1. Choice of Mutagen

A long held notion, which probably has been influencing the choice of mutagen types (chemical vs. physical), is that chemical mutagens preferentially induce point mutations while physical mutagens induce gross lesions, such as chromosomal breakages and rearrangements. This view has probably been aided by the relative ease for procuring chemical mutagens as well as simpler set-up requirements. Current empirical data do not support this conclusion; rather the more plausible inference from available information is that the frequency and types of mutations are direct results of the dosage and rate of exposure or administration of the mutagen rather than its type. This implies

therefore that the extended immersion of plant propagules in high concentrations of EMS or NaN_3 would be equally as deleterious or even lethal as extended exposures to high doses of ionizing radiation. In all cases, optimal doses of mutagens should always be determined prior to large-scale induction of mutations. It should also be pointed out that chemical mutagens are very toxic poisons that usually require elaborate detoxification of laboratory ware and disposal of used reagents. Relatively, the risks to health posed by physical mutagens are far less. In the end, the choice of a mutagen will be based on the particular researcher's circumstances such as the availability of the mutagens, associated costs and available infrastructure.

3.2. Practical Considerations in Induced Crop Mutagenesis

Mba *et al.* [39] provided the protocols for the induction of mutations in seed and vegetatively propagated plants, based on procedures validated for rice and cassava, respectively. The protocols were for the physical mutagen (gamma rays generated by a cobalt-60 source) and the chemical mutagen (ethyl methane sulfonate, EMS). The authors [39] listed the required equipment and reagents and described the sequential procedures, the pre-treatment handling of the propagules, the mutagenic treatments, post-treatment handling of the materials; and the methodologies for the collection and analysis of data. In general, the steps to the induction and detection of mutants vary between seed and vegetatively propagated crops but would nonetheless involve the following considerations:

- The setting of realistic targets matched with the availability of the requisite appropriate human and material resources and the prior determination that induced crop mutagenesis offers a comparative advantage over other available technologies and strategies.
- A clear understanding of the genetics of the traits to be improved; for instance, polygenic traits (*i.e.*, characters under the influence of many genes) have less chance of being modified with induced mutations than monogenic traits (*i.e.* characters under the influence of single genes).
- A knowledge of the reproductive biology of the crop, *i.e.*, seed *vs.* vegetative propagation; if seed propagated, self- *vs.* cross-fertilization; and if vegetatively propagated, which propagation methods are possible (*in vivo vs. in vitro*).
- The determination of the plant propagule to be treated *i.e.* botanical seeds or gametes for seed propagated crops and nodal segments, stem cuttings, twigs, buds, or different types of *in vitro* cultures for vegetatively propagated crops and the assemblage of sufficient quantities.
- A knowledge of the ploidy levels of the crop especially when ploidy-related hybridization barriers could impact on the envisaged utility of the induced mutants.
- A determination of the genetic background of the target materials for induction of mutations. Usually, the best genetic background to be induced to mutate should in general be the best all-round genotype that is deficient in a single trait (the breeding objective); and is homozygous.
- An informed decision on which induction method, *i.e.*, physical *vs.* chemical mutagens, and the appropriate doses (including considerations of concentrations and durations for chemical mutagens). A pilot assay is usually necessary in order to determine the optimal dosage prior to the large-scale bulk treatment of the propagules.

- A clear definition of the plans, mechanisms and the identification of the requisite facilities for handling (including for planting and evaluating) the mutant population. This would also involve an understanding of, and planning for, the adequate population sizes.
- The scheduling, as the case may be, for the use of the identified infrastructure (irradiator, screen house, fields, laboratories, *etc.*) and other required inputs.
- As may be necessary, a definition of methodologies for the identification of the mutation events and subsequent selection of desired mutants. Options may include molecular biology assays with techniques that lend themselves to high throughput set-ups being preferred since they generally obviate the drudgery associated with handling large mutant populations, the majority of which will be jettisoned.
- Methodologies for the dissociation of chimeras and the production of stable mutants must also be clearly thought through. For putative mutants destined for plant breeding programs, a clear definition, along with relevant counterparts, of the means for incorporating the stable mutants into hybridization schemes is critical to success.

The important experimental factors that influence the outcomes of chemical mutagenesis include the concentration of mutagen; volume of mutagen relative to size of samples; treatment duration; temperature; pre-soaking of seeds; pH; use of catalytic agents; and the post-treatment handling of the putative mutants [39]. For physical mutagens, the authors [39] identified the presence of elements such as oxygen and chemical mutagenic agents; moisture content; impurities such as dust and fibers; and viral and bacterial infectious agents as being critical to the outcome. The determination of the optimal doses of mutagens and the dissociation of chimeras in putative mutants are probably the most critically important of factors for the success of induced mutations.

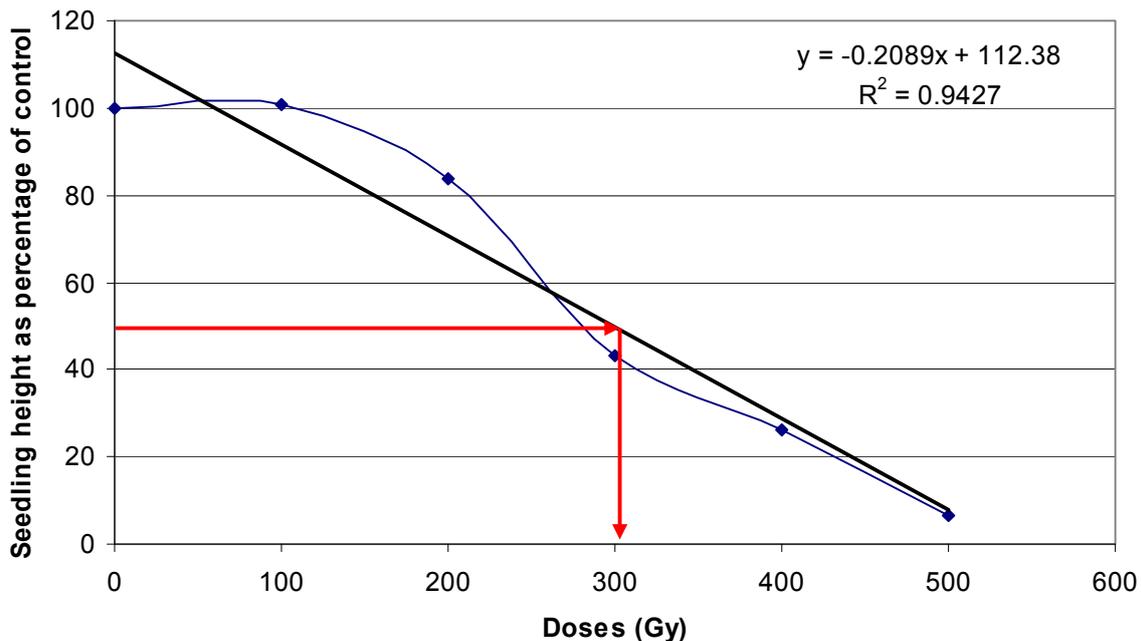
3.3. Optimal Doses for Mutagenic Treatment

For crop breeding purposes (as against generating mutant stocks for functional genomics, for instance), the aim should be to obtain a reasonable number of desired mutations for a trait of interest while inflicting the least unintended disruption to the genotypic integrity of the crop. This ensures that the fitness of the desirable induced mutant is not otherwise compromised by the presence of unintended induced deleterious alleles which would require additional interventions in time and resources for, e.g., backcrossing to the elite starting genotype in order to break linkage drags.

The universally adopted norm is to select a dosage that results in reductions of 30 to 50 or 40 to 60 percent in growth or survival rates, respectively of the first generation mutant (M_1) seedlings compared to the seedlings of untreated seeds. Reductions in germination rate, seedling height, survival rate, number of tillers, seed set, and fertility test in the M_2 generation as well as chlorophyll mutation are the main parameters measured in the sensitivity tests for determining the optimal doses. The reductions are plotted against the mutagen doses and the dose corresponding to the desired level of reduction is read off the graph. This could also be determined through regression analysis. A sample graph, Figure 1, shows that a 50% reduction in seedling plant height is caused by gamma irradiation at 300 Gy. Doses corresponding to other percentage reductions can be read off the gradient of the graph. Mba *et al.* [39,40] described the procedures for using the radiosensitivity to the physical mutagen,

gamma rays, and the reactions to EMS to ascertain their optimal doses for inducing mutation events in both seed and vegetatively propagated plants.

Figure 1. Percentage of reduction in plant height of seedlings germinated from seeds exposed to a mutagen (compared with seedlings from untreated seeds), plotted against mutagen dosage. The red arrows indicate that seedling height reduction of 50% is caused by 300 Gy.



3.4. Incidence of Chimeras

Usually, multi-cellular tissues are used as the starting material for mutation induction. Following induced mutagenesis, the individual cells of putative mutants typically vary amongst themselves in terms of the induced mutations that they harbor. This is chimerism, *i.e.*, sectoral differences in a mutant, whereby cells of different genotypes exist side-by-side in the tissues of the same individual plant. In essence, the plant consists of genetically distinct somatic tissues. Chimeras are significantly dissociated in seed propagated crops in one to two generations of selfing (M_2 to M_3) while for vegetatively propagated crops, several generations of vegetative propagation are required to produce solid homohistonts. Along with the absence of meiotic sieves, the fixation of deleterious alleles, the transmission of pathogens to subsequent generations, the incidence of chimeras are considered the most serious drawbacks to the induction of mutations in vegetatively propagated plants [40,41]. Considerable efforts are currently being invested in the determination of strategies for exploiting totipotency, *i.e.*, the ability of individual single plant cells to regenerate a whole organism, to quicken the pace of chimera dissociation in induced mutants. Some of the cell and tissue biology techniques that enable this are discussed in subsequent sections.

3.5. Induced Mutants as Raw Materials for Crop Improvement

Following the demonstrations of the mutagenicity of X-rays on fruit flies and maize by Muller [16] and Stadler [18], respectively, plant scientists devoted significant efforts to establishing the experimental protocols for the use of radiation to generate heritable variations that could be used in crop improvement. Within three decades, mutation breeding had become an established plant breeding strategy and in a little less than a century is credited with the development of over 3200 crop varieties that are being grown all over the world [42]. The success of plant breeding depends largely on the availability of utilizable heritable variations. When desirable variations are easily discernible from well characterized germplasm collections, the plant breeder's task is fairly straightforward. In instances where such variations are either unavailable to the breeder or are observed only in materials with otherwise undesirable genetic backgrounds, induced mutations may be the only way to generate desirable variations for use in breeding superior crop varieties.

The Joint Division of the Food and Agriculture Organization of the United Nations and the International Atomic Energy Agency (Joint FAO/IAEA) in Vienna, Austria which maintains a Mutant Varieties Database (MVD) for these crop varieties [42] introduced some newly developed mutant rice varieties in China and Bangladesh on its website [43]. For China, these were the rice variety with modified starch characteristics suitable for managing diabetes and obesity; one with high contents of zinc; and yet another with round grains of reduced size that is suitable for formulating baby food. The newly released high yielding rice variety in Bangladesh, BINA Dhan-7, matured earlier than other varieties of comparable yield by about one month, was flood and disease resistant and also amenable to intercropping. These varied induced variations are reflective of the other over 3200 crop varieties that have been developed through induced mutation-mediated strategies in the over 80 years since the demonstration of the feasibility of induced mutations for crop improvement [42].

Most, about 77%, of these mutant crop varieties are seed propagated probably underscoring the relative ease for the induction of mutations using botanical seeds as against stem cuttings, tubers and other vegetative propagules, as starting materials for induced mutagenesis. It might also be a reflection of the preponderance of seed propagated crops (cereals and pulses) as the major food crops of the world. Almost half (48%) of all mutant crop varieties recorded in the MVD are cereals. Rice is the crop with the highest number of mutants and accounts for 53% of the mutant cereals under cultivation followed by barley which makes up 20% of all cereal mutant varieties globally. Figures 2 and 3 show the relative distributions of these mutants by the continent where they were developed and crop usage type, respectively. More than half (60%) of the mutant crop varieties have been released in Asia. China alone accounts for more than 25% of all mutant varieties that have been officially released globally. Interestingly, China along with two other Asian countries, Japan and India are the top three countries with the most officially released mutant crop varieties. These trends are largely unchanged from the observations of [44] who chronicled the over 2200 officially released mutant varieties by the year 2000 while [45] provided an in-depth review of the contributions of both spontaneous and induced semi-dwarfness to the genetic improvement of the cereals, rice, bread and durum wheats and barley, especially in the context of the Green Revolution.

Figure 2. Distribution of mutant crop varieties reported on the Joint Division of the Food and Agriculture Organization of the United Nations and the International Atomic Energy Agency (Joint FAO/IAEA) Mutant Varieties Database [42] by continents of official release.

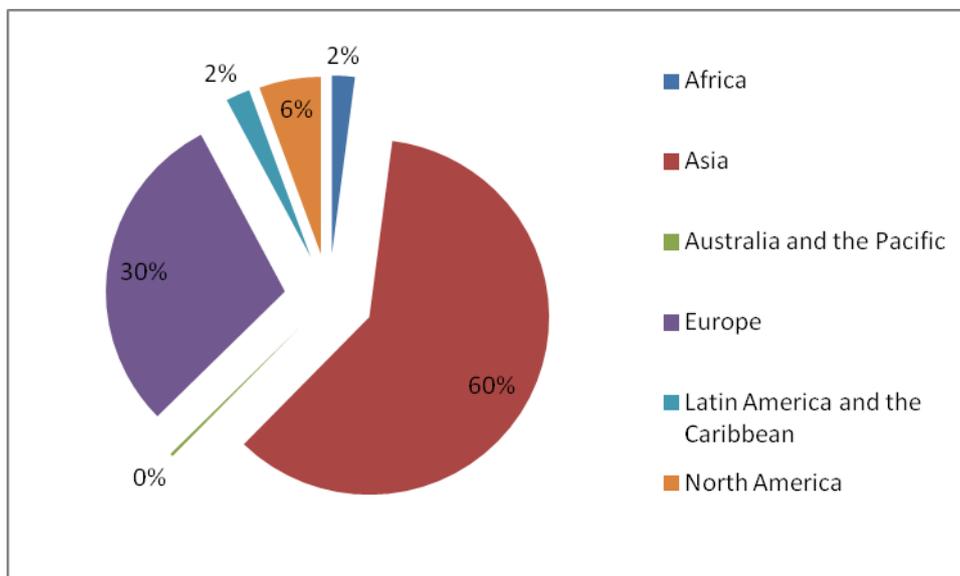
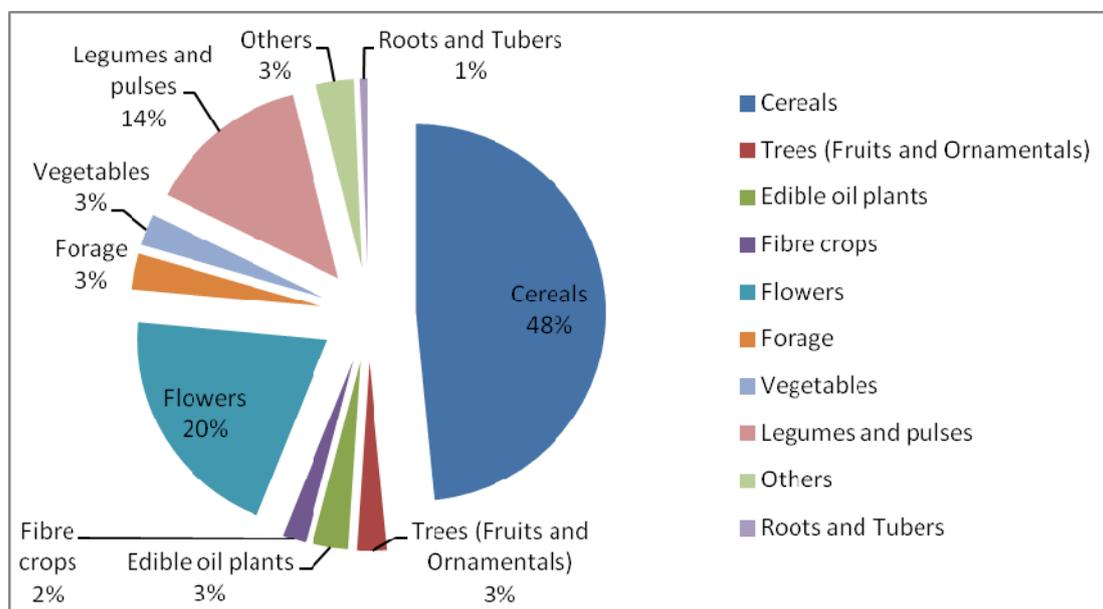


Figure 3. Distribution of officially released mutant crop varieties reported on the Joint FAO/IAEA Mutant Varieties Database [42] by crop type and usage.



Ahloowalia *et al.* [46] surveyed the economic importance of some of the outstanding mutant crop varieties globally based on such indices as annual acreage; estimated reduction in the use of agricultural inputs (on account of improved hardiness); synergistic increases in yield; enhanced market values (on account of improved quality traits); increase in export earnings for the farmers; reduction in food importations; *etc.* These included varieties of rice in Australia, China, India, Pakistan and Thailand; cotton and wheat in Pakistan; pear in Japan; grapefruit, sunflower and peppermint in the USA; barley in several countries of Europe; durum wheat in Italy; sorghum in Mali; groundnut and

pulses in India; and several ornamental plants in India, the Netherlands and Germany. Taking barley for instance, the authors [46] reported that the high-yielding and dwarf mutant cultivars of barley “Diamant” and “Golden Promise” had jointly added billions of dollars in additional income to the brewing and malting industries. According to the authors [46], more than 150 leading barley cultivars in several countries in Europe, North America and Asia were derived from crosses involving Diamant. Over 20 million US dollars in additional income accrue to farmers per annum on account of growing these two barley varieties and their progenies making them therefore the mainstay of Europe’s malting industry.

Ahloowalia *et al.* [46] provided other examples of the benefits accruing from the cultivation of other notable mutant crop varieties; these included:

- Most varieties of durum wheat grown in Italy and used in making pasta that is marketed worldwide are induced mutants; their cultivation generates tens of millions of dollars in additional income to farmers and the seed industry per annum;
- The Rio Star variety of grape fruit in the USA is a mutant that accounts for 75% of the US grapefruit industry;
- The cultivation of the mutant Japanese pear variety, “Gold Nijesseiki”, contributes US\$30m in additional income to farmers annually
- A mutant cotton variety, NIAB78, grown widely in Pakistan accounts for over US\$20m in additional income to farmers annually
- Most of the rice grown in Asia (especially in China, Japan, Vietnam and India) and Australia are mutants. The additional income to farmers for growing and marketing these rice varieties is estimated in billions of US\$ annually
- Mutant barley varieties with enhanced adaptation to extremely harsh environmental conditions in high altitudes are literally extending the frontiers of arable lands in Peru. Their cultivation provides much needed employment for resource-poor farmers.

More recently, Kharkwal and Shu [47] reviewed the contributions of induced crop mutants to global food security by highlighting some of the important varieties developed through induced mutagenesis mediated-strategies, their annual acreages in the countries of cultivation and some other indices that underscore their importance in food security and income generation. The crops covered in this review included rice in China, Thailand, Vietnam, and the USA; barley in European countries and Peru; durum wheat in Bulgaria and Italy; wheat in China; soybean in China and Vietnam; and some other food legumes in India and Pakistan. They concluded that there was considerable evidence that mutant crop varieties would continue to contribute significantly to addressing the food and nutritional securities of many countries especially in view of the potentials for harnessing novel traits in enhancing the adaptabilities of crops to climate change and variations.

A crop variety with significant economic potentials that has been developed recently through induced mutations is linola, the trade name for the edible linseed crop, developed from the non-edible linseed flax, *Linum usitatissimum*, by the Australian Commonwealth Scientific and Industrial Research Organisation [48,49]. With mutations to the fatty acid synthesis pathway, the alpha-linolenic acid (ALA) content was reduced to 2% (as against 50% in the wildtype). Oil produced from the wildtype becomes rancid quite rapidly on account of this high content while linola oil is comparable to the oils

from sunflower and canola. Linola is now being produced in Australia, Canada, the United Kingdom and the United States. Interestingly, canola was developed exploiting spontaneous mutations that lowered the levels of erucic acid and glucosinates, both anti-nutritional factors found in the wildtype rapeseed or field mustard.

Mutant crop varieties therefore form integral parts of daily diets all over the world; are raw materials in industries; and contribute billions of dollars in additional income to farmers. The traits that confer superiority on the mutants over other cultivars include synergistic higher yields due to enhanced resistances to biotic and abiotic factors; improved nutrient use efficiencies; and nutritional and other quality traits. In being hardier and more nutrient use efficient and therefore requiring less agricultural inputs, several mutant crop varieties are more economical to grow and contribute to more environment friendly agriculture.

3.6. Induced Mutants as Tools for Understanding and Harnessing Heredity

The use of characterized mutants in plant breeding has long been an established practice. But interestingly, the observed resurgence in induced mutagenesis in plants (after a lull that commenced in the 1980's in the wake of rapid epochal advances in recombinant DNA technologies), seems to be mostly on account of increased investments in the production of genetic resources for functional genomics, *i.e.* the identification genes and the elucidation of their functions. With the ever increasing ease for obtaining DNA sequence information and even sequencing whole genomes, massive amounts of information on the genomes of many organisms have become publicly available. The deciphering of the information coded into the sequences has not been as routine, however.

Taking advantage of these massive troves of genomic information that is increasingly available for a number of plant species, induced mutants are used in identifying and ascribing functions to genes. The genomic region(s) implicated in the expression of a trait, *i.e.*, the gene, is identified by studying a series of induced mutants vis-à-vis the normal or wildtype variants. Adopting the deductive process of identifying which traits have been modified and relating the modifications to changes in genomic regions of the induced mutants, as compared to the normal types, the gene(s) controlling a trait may be identified.

A significant part of the current efforts and resources invested in induced mutagenesis in plants (including the systematic molecular and morphological characterizations of mutant collections) is devoted to functional genomics. For a majority of the model plants and other intensively researched plant species, including major crop plants, huge amounts of genotypic and phenotypic data are being generated and housed in publicly accessible searchable databases. Typically, researchers rely on such information to request the characterized mutants for scientific investigations. Examples of plant mutant stocks that are publicly available to requestors include the model plant, *Arabidopsis*, maize, rice, barley, peas, cucurbits and tomato (Table 4). The U.S. Department of Agriculture, Agricultural Research Service (USDA/ARS) has a number of germplasm collections that hold publicly available characterized mutant stocks under the National Plant Germplasm System (GRIN) [50]. A number of public germplasm collections in other countries also have substantial number of mutant accessions that constitute a critical resource for the continually increasing plant functional genomics research community. Some of these mutant stocks are listed in Table 4.

Table 4. Some characterized mutant stocks of crops and the host institutions.

Crop	Host institution	Reference
Maize	The Maize Genetics Cooperation Stock Centre, University of Illinois, Urbana/Champaign, IL, USA	[51]
Arabidopsis	European Arabidopsis Stock Centre (or Nottingham Arabidopsis Stock Centre, NASC), University of Nottingham, Sutton Bonington Campus, UK	[52]
	Arabidopsis Biological Resource Centre, (ABRC) Ohio State University, OH, USA	[53]
Tomato	CM Rick Tomato Genetics Resource Centre, University of California at Davis, CA, USA	[54]
Cucurbits (cucumber, melon, <i>Cucurbita</i> , and watermelon)	Cucurbit Genetics Cooperative (CGC) North Carolina State University Raleigh, NC, USA	[55]
Rice	The Oryzabase of the National BioResource Project— Rice National Institute of Genetics, Japan	[56]
	IR64 Rice Mutant Database of the International Rice Functional Genomics, International Rice Research Institute, Manila, Philippines	[57]
	Plant Functional Genomics Lab., POSTECH BIOTECH CENTER, San 31 Hyoja-dong, Nam-gu Pohang, Kyoungbuk, Korea	[58]
Barley and wheat	Barley mutants, Scottish Crop Research Institute, Dundee, Scotland	[59]
	Barley and Wheat Genetic Stock of the USDA-ARS USDA-ARS Cereal Crops Research Unit, Fargo, ND, USA	[60] [61]
	Wheat Genetics Resource Center, Kansas State University, Manhattan, KS, USA	[62]
	Wheat Genetic Resources Database of the Japanese National BioResource Project	[63]
Pea	Pea mutants, John Innes Centre, Norwich, UK	[64]

3.7. Enhancing the Efficiency for Induced Mutagenesis

Major drawbacks to induced mutagenesis include the requirement for generating large mutant populations, the incidence of chimeras and the heterozygosity of the mutated loci. By integrating cell and tissue biology techniques and molecular biology strategies in the induction and detection of mutations, these constraints are mitigated without compromising the imperative of generating large mutant population sizes. The judicious integration of these novel biotechnologies permits the rapid generation of large mutant populations with desired genetic backgrounds; the targeted querying of specific regions of the genome that control a trait of interest for induced alterations. The following are some molecular biology and cell biology techniques with demonstrated potentials for enhancing the efficiency of induced crop mutagenesis.

3.7.1. Reverse Genetics

The classical genetics approach that relies on the comparing of the phenotypic expressions of variants (spontaneous or induced mutants) with those of the wildtypes in order to identify and characterize the causal genes is known as forward genetics. With the current advances in genetics—the discovery of DNA, the polymerase chain reaction and genome sequencing, for instance—it is now possible to alter or disrupt a specific gene or gene product (whose effect may be previously unknown) and then determine the phenotypic effect of such a modification. In essence, rather than working from the phenotype to the genotype, scientists can now work backwards, from the genotype to the phenotype; this is known as reverse genetics. The reverse genetic technique, TILLING (Targeting Induced Local Lesions IN Genomes [65], that was aptly characterized “Traditional Mutagenesis Meets Functional Genomics” [66], permits the high throughput querying of putative mutants for point mutation events in specific genomic regions. With TILLING, the mutation events are identified by enzymatic cleavage of mismatches between a mutated strand and a wild type one. It therefore has huge potentials for removing the major bottleneck to the routine application of induced mutagenesis: the need to produce, handle and query large putative mutant populations of sufficient mutation density for low frequency recessive events. Since the proof of concept about twelve years ago [65], TILLING has been successfully applied in the detection of mutations in several plant species. These include *Arabidopsis* [67–70]; rice [71–74]; maize [75]; wheat [76,77]; sugar beet [78]; barley [79]; soybean [80]; pea [81]; beans [82,83]; tomato [84] and in the vegetatively propagated banana [85]. The related technique, Ecotilling, is also a robust method for identifying spontaneous mutants and hence useful for characterizing germplasm in general [86]. Initially optimized with the chemical mutagen, EMS, TILLING has also been effective in identifying point mutations induced by physical mutagen [87]. The TILLING innovators [65] used denaturing HPLC for detecting the mutations. This methodology lacked the high throughput functionality needed for handling large mutant populations. To redress this, a more robust and high throughput platform that ran on LI-COR gel analyzer system (Lincoln, NE, USA) with an analytical software was described by Colbert *et al.* [68]. This method became widely applicable and was the platform for using TILLING to identify mutants in the above plant species but more recently, Tsai *et al.* [88] have described an alternative, TILLING by sequencing. Rather than use endonuclease to cut DNA strands at heteroduplexes produced by the mismatch between wildtype and mutant strands, the next generation sequencer Illumina is used to sequence target genes from pooled DNA samples. Till *et al.* [89] surveyed some ongoing TILLING projects and their activities; a number of these facilities also provided mutation discovery services to requestors. Table 5 shows some of the facilities providing TILLING services.

Table 5. Some research and development facilities offering TILLING services.

Plant	TILLING Facility	Reference
Rice, tomato, <i>Arabidopsis</i>	The UC Davis TILLING Core, University of California, Davis, CA, USA	[90]
Tomato	Metapontum Agrobios Centre, Metaponto di Bernalda, MT, Italy	[91]
Maize	The Maize TILLING Project, Purdue University, West Lafayette, IN, USA	[92]
Brassica napus	CAN-TILL, University of British Columbia, RevGenUK, John Innes Centre, Norwich	[93]
Lotus, Medicago, Brassica rapa	Research Park, Colney, Norwich, NR4 7UH, UK	[94]
Arabidopsis	Seattle TILLING Project, Fred Hutchinson Cancer Research Center, North Seattle, WA, USA	[95]
Tomato, Rapeseed, Pea, Medicago and Melon	Plant Genomic Research, INRA/CNRS—URGV, Evry cedex, France	[96]

3.7.2. Cell and Tissue Biology

The very low levels of efficiency for the induction and detection of mutation events and the production and evaluation of large putative mutant populations constitute significant hindrances to the routine application of induced mutations in plants for crop improvement and functional genomics. Large populations of induced mutants must be produced in order to enhance the chances for identifying the low frequency and largely recessive induced mutations. Equally confounding efforts in induced mutagenesis is the imperative of dissociating chimeras, a problem that is more pronounced with vegetatively propagated plants. Also problematic is the need for homozygosity of the mutated loci so that the induced mutation, usually recessive, could translate to a phenotype. The efficacies of some *in vitro* techniques in mitigating these constraints significantly have been demonstrated. Some proven methodologies are the incorporations of cell suspension cultures, including somatic embryogenesis; doubled haploidy and rapid *in vitro* multiplication techniques into the processes for the induction, detection and evaluation of mutation events.

3.7.2.1. Cell suspension cultures

The incidence of chimeras in putative mutants is a major problem in induced mutagenesis especially with vegetatively propagated plants on account of the multicellular vegetative propagule which lacks the corrective effects of the meiotic sieve. Chimeras are dissociated by subjecting the putative mutants to several cycles of vegetative regeneration (both *in vitro* and *in vivo*). This, of course, introduces added expense in time and resources. The use of cell suspension cultures as starting materials for inducing mutations such that single cells are mutagenized would be a means to avoid the additional expense and investments in time and labor. The efficacy of cell suspension cultures derives from the potential of each plant cell to regenerate into a whole plant; this is the phenomenon of totipotency. For cell suspension cultures, cell lines are produced from callus followed by the

regeneration of plantlets through somatic embryogenesis. In practice, cell suspension involves the culturing *in vitro* of single cells and small cell aggregates; these proliferate and complete a growth cycle while suspended in liquid medium. Ever since the pioneering work of Nickell [97] with *Phaseolus vulgaris*, the production of cell suspension cultures has become routine for many plant species. It holds great promise therefore for induced mutagenesis in that the incidence of chimeras in plants arising from mutagenized single cells would be quite minimal. This would result in significant savings in the time that would ordinarily be invested in the several cycles of regenerations that would ordinarily be required to dissociate chimeras. The pilot testing of this concept with *Musa* has been promising [98].

3.7.2.2. Doubled Haploidy

Another bottleneck to the exploitation of induced crop mutations is the predominantly recessive nature of mutations. The consequence of this is that the expression of the desired phenotype is masked if the alleles are in a heterozygous state. Attaining homozygosity at the mutated alleles so that the desired phenotypes can manifest requires several cycles of selfing and hence added expense. Again, totipotency is exploited to produce doubled haploids (DHs), *i.e.*, whole plant that arise from the doubling of the chromosome number of gametic cells, pollens and egg cells, prior to their regeneration [99,100].

There is considerable promise for the routine incorporation of doubled haploidy into induced mutagenesis by treating these gametic cells prior to regeneration of the DHs [101–103]. With the availability of *in vitro* mechanisms that permit the spontaneous and/or induced doubling of the haploid chromosomes, homozygous individuals could be produced quite routinely [104]. This would be a very rapid route to homozygosity [105,106] of the mutant's genome and hence the mutated allele. Indeed, the authors [106] concluded that a mutation is captured in a homozygous, pure line in just one generation by using either the haploid or doubled haploid cells as starting materials for mutation treatments. This implies that the gametes of the M_1 plants could be subjected to doubled haploidy or the gametes (haploid cells) of the wildtypes could be induced to mutate and then subsequently subjected to doubled haploidy. Both routes achieve the same purpose of the attainment of homozygosity in one generation. Szarejko and Forster [106] reviewed the productions of DH from M_1 materials of *Hordeum vulgare*, *Oryza sativa*, *Solanum nigrum* and *Triticum aestivum*. Mutations had also been induced successfully in haploid cells of different species of *Brassica*, *Datura innoxia*, *Hordeum vulgare*, *Malus x domestica*, different species of *Nicotiana* and *Oryza sativa*. Different physical and chemical mutagens were used. For seed propagated crops therefore, DH strategies provide the fastest method for attaining homozygosity. The savings in time and cost are significant as recessive mutations usually are not detectable till the second (M_2) or later generations of selfing. Reproducible DH protocols are available for over 250 plant species [107] across most plant genera. Technical challenges still remain however with regard to developing genotype-independent protocols, improving the level of callus production by the pollen grains, the differentiation of callus into green plants and the doubling of chromosomes.

4. The FAO and IAEA Partnership for Nuclear Techniques in Agriculture

FAO and the IAEA have for over 45 years through their Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture headquartered in Vienna, Austria partnered to support countries' efforts to attain food security [108]. The Plant Breeding and Genetics Section of this program assists countries in using radiation induced mutations, facilitated by biotechnologies, to develop superior crop varieties [108]. The Section implements its mandates through a combination of field projects in developing countries; the coordination of collaborative research networks; and a research and development laboratory arm in Seibersdorf, outside Vienna. Currently, there are a total of 86 field projects with themes relating to induced crop mutations (Table 6). The main thrust of the field activities, known as Technical Cooperation Projects (TCP), is technology transfer characterized by the strengthening of human and infrastructural capacities. The irradiation facilities, mostly cobalt-60 sources, in many countries were provided through this TCP mechanism. A more upstream intervention of this partnership is the Coordinated Research Project (CRP) mechanism which fosters global networking between institutions that pool efforts and resources in addressing a mutually important research problem [109]. There are currently six of such CRPs with themes pertaining to induced crop mutations and ancillary biotechnologies [110]. These interventions are complemented by the adaptive research on similar themes carried out its laboratory in Seibersdorf Austria [111]. Training and the provision of irradiation services also form part of the core activities of the laboratory.

Table 6. Ongoing field projects relating to induced crop mutations managed by the Joint FAO/IAEA Program.

Geographical Location	Number of Projects		
	National	Regional	Total
Africa	34	2	36
Asia and the Pacific	29	8	37
Europe	2	2	4
Latin America and the Caribbean	7	2	9
Total	72	14	86

5. Emerging Trends

The case has been made that in order to become an effective and efficient complement to the suite of modern crop improvement and functional genomics strategies, a significant amount of the associated drudgery must be mitigated. This desired efficiency can be achieved by the incorporation of the very powerful novel biotechnologies that have significantly amplified the scopes for addressing biological questions into the processes for the induction, detection and deployment of mutation events. Genomics, the study of the whole genetic makeup of an individual at the molecular level, is particularly suited to the enhancing of efficiencies of the detection and deployment of mutation events. To fully realize the benefits of induced mutagenesis however, there must also be reliable means for generating phenotypic data at high throughputs. A means for effectively injecting the mutants, despite the deleterious alleles that they additionally carry, into breeding programs must also be well thought through. Clearly, the bottlenecks to phenotyping large mutant populations must be removed just as the

additional costs for breaking linkage drags must be reduced implying therefore that phenomics and pre-breeding will become increasingly important ancillary aspects to induced mutagenesis.

5.1. Phenomics

Granted, the amenability of genomics platforms to high throughput processes permits the simultaneous generation of very reliable genotypic data from multiple samples and hence abates the major drawback to induced mutations: the imperative of generating and evaluating large numbers of putative mutants in quest of invariably low frequency events. But, for the genotypic data to lead to valid inferences on the variants, there must be reliable mechanisms for generating the complementary phenotypic data. The routine adoption of phenomics, defined by Houle *et al.* [112] as the “the acquisition of high-dimensional phenotypic data on an organism-wide scale” as a component of the detection and deployment of mutation events holds immense promise in this regard. Phenomics facilities typically make use of thermal infra-red, near infra-red, fluorescence and even magnetic resonance imaging equipment to collect large amounts of physiological, morphological and biochemical data from parts of live plants in very short periods of time. Phenomics is permitting a greater understanding of the mechanisms that govern such complex traits as drought and salinity tolerances [113,114]. The set up of phenomics facilities is undoubtedly an expensive endeavor that may be unaffordable for many national agricultural research organizations but the availability of service providers in Australia [115]; Canada [116]; France [117,118]; and Germany [119,120] might serve as impetus for setting up other such facilities to cater to scientists in different parts of the world. The CGIAR centers and other centers of excellence for crop-related agricultural research could incorporate phenomics into their activities and hence serve as regional hubs. The Joint FAO/IAEA laboratory that traditionally provides services relating to induced mutations is also well positioned to take on this additional task.

5.2. Pre-Breeding

The extremely narrow genetic base of the world’s important crops [2,121] is a major cause for concern as efforts are invested to crop productivities and enhance the resilience of cropping systems under climate change regimens. It is ironic therefore that plant breeders largely continue to rely on the same “safe bet” parents in breeding programs. Many reasons, amongst which is the avoidance of the extra expense in time and resources required for breaking the linkage drags associated with the introgression of traits from crop wild relatives, landraces and other otherwise non-adapted materials, have been adduced for this predilection. The same mindset dampens the enthusiasm for generating induced mutants and incorporating them into crossing blocks. Unintended and undesirable induced mutations typically co-segregate with the desirable novelties, a “nuisance factor” that is expensive to get rid of. Pre-breeding, described by Nass and Paterniani [122] as “all activities designed to identify desirable characteristics and/or genes from unadapted (exotic or semi-exotic) materials, including those that, although adapted have [not] been subjected to any kind of selection for improvement” is a means to populate the gap in activities between curating germplasm collections and plant breeding with the generation of intermediate breeding materials. The Global Crop Diversity Trust elegantly describes this collaborative discipline between the germplasm curator and plant breeder as “... the art

and science of identifying desired traits in otherwise unprepossessing and unpromising plants, often wild, and starting to incorporate them into modern varieties” [123].

For practical purposes, the raw putative induced mutants may be considered as those “otherwise unprepossessing and unpromising plants” that must be put through the paces of selections and hybridizations in order to elevate them to the stable and relatively “clean” materials that may be used as parents in breeding programs. The e-learning course [124] that was developed by the Global Partnership Initiative for Plant Breeding Capacity Building is a very useful tool for assisting germplasm curators (with in-depth knowledge and appreciation of the variations in germplasm collections) and the plant breeders (who work to incorporate the most desirable new traits into novel varieties) institutionalize this paradigm for crop improvement. It includes modules on germplasm management, classical plant breeding themes and molecular genetics techniques and therefore imparts skills that lend themselves to managing large mutant populations and integrating their novel traits into breeding lines. Pre-breeding, therefore, is indeed a most valuable appendage to all induced mutation programs as resources could be dedicated solely to transforming these “raw” induced mutants with all the deleterious alleles to “acceptable” parental materials for breeding programs. It is safe to assume that as pre-breeding becomes an established component of crop improvement, plant breeders will be under less pressure to account for the time invested in cleaning up induced mutants for use as parents in hybridization. Induced mutation projects should therefore include a pre-breeding component as means to forge the bridge to plant breeding.

6. Conclusions and Future Perspectives

Enhanced productivities on farmers’ fields, with minimal ecological footprints, is a sustainable means for addressing the myriad constraints that threaten the 21st century global food security. Clearly, a diverse set of hardy, high yielding and input use-efficient crop varieties will, along with ecosystem-based agronomic practices, constitute the crop production packages needed to nourish an ever increasing human population, meet the demands of other industries for food-based substrates and yet leave the environment largely unharmed. The currently available cultivars of most staple crops do not fit into this envisaged highly efficient yet low-input crop production systems. This implies that a new portfolio of crop varieties will need to be bred.

Breeding the needed novel varieties will require a most judicious application of the most appropriate scientific and technological tools so that the inherent potentials of PGRFA are harnessed optimally [14,125]. Induced mutagenesis, albeit almost a century old technique, demonstrably can contribute to unleashing the potentials of PGRFA and thereby avail plant breeders and other scientists the raw materials needed to generate the envisaged “smart” crop varieties. Induced mutagenesis is a proven, safe, robust and cheap plant breeding strategy. Crop varieties generated through the exploitations of induced mutagenesis are contributing to global food and nutritional security and improved livelihoods. But, induced mutagenesis, as a crop improvement strategy, has for long been an inefficient means for accessing novel alleles of genes. This is largely on account of the requirement for generating and evaluating large populations of putative mutants, the incidence of chimeras and the recessive nature of mutations. This need not remain the *status quo* as the ever increasing access to publicly available genomic and genetic resources along with other advances in high throughput

molecular genetics, cell biology and phenotyping techniques are proven means to mitigating these constraints and hence, enhancing efficiencies. These novel techniques facilitate the integration of induced mutations into seamless processes for crop improvement and classic genetics research.

Also, by inducing crops to mutate, the ability of scientists to understand and better exploit the underlying genic influences that modulate the expression of agronomic and crop quality traits is greatly enhanced. Induced mutagenesis is therefore now a strategy of choice in functional genomics as it greatly facilitates the identification of genes and the elucidations of their functions. Interestingly, the outputs of functional genomics, namely, elucidated genes, are, when used as molecular genetic markers, enhancing the efficiency of plant breeding (including mutation breeding). It is also noteworthy that phenomics will facilitate the generation of morphological and/or agronomic data or those of their contributory parameters. In like manner, pre-breeding will enhance the ease for integrating mutants into breeding programs. The model of the Joint FAO/IAEA Program, in being dedicated solely to the deployment of “atoms for peace” in food and agriculture, is innovative and unique. It is currently supporting R&D activities, providing training and niche services, facilitating technology transfer and fostering communities of practice. This model is deserving of emulation and scaling up.

In spite of the aforementioned advantages of, and benefits accruing from, induced mutations, it is hardly a mainstream endeavor in crop improvement. A majority of plant breeding programs have no active mutation induction activities while a number of irradiation facilities have been closed in the last two to three decades. It is plausible to infer that the decline in the applications of induced mutations—which parallels the general decline in capacities for crop improvement, especially, plant breeding—correlates inversely with the upsurge in the adoption and applications of biotechnologies, especially recombinant DNA technologies. But, it need not be one or the other being as the techniques are vastly different. Induced mutations, for instance, does not entail the introduction of extraneous hereditary materials which subsequently express in the recipient genome. This alone exempts induced mutants from the often times expensive and long regulatory regimes that genetically modified organisms are subject to. This relative simplicity of official processes couples with the earlier mentioned robustness and cheap start up and operational costs to make induced mutations particularly suitable for developing countries.

However, for induced mutagenesis to once again become a mainstream crop improvement technique for the 21st Century, significant efforts must be invested in a number of interventions. These include:

- Training of the new crop of plant breeders. Induced mutations, along with enabling biotechnologies, ought to be part of university curricula at both the honours and graduate levels. The annual training program of the Joint FAO/IAEA Division in induced crop mutations could provide the template for developing course curriculum that would be adaptable to other situations. In the same vein, students already undergoing formal plant breeding training programs in universities could also be deliberately targeted for inclusion in this training course. Equally, universities that have maintained strong programs in induced mutations may be supported to host trainees on ad-hoc basis.

- Research. Granted, biotechnologies, such as reverse genetics and cell biology, facilitate the induction, detection and transfer of desirable mutation events. But, the protocols, most of which are genotype-dependent, have been developed for the well-researched major crops. More investments are clearly required for adapting the protocols or developing new ones for the “orphan” crops that are the mainstay of the cropping systems of many food insecure countries. Easily adaptable protocols for vegetatively propagated crops are particularly in short supply. Also, protocols that are less resource-intensive and are adaptable to the resource-poor research environments in developing countries are needed. For instance, for TILLING, neither the endonuclease cleavage of heteroduplexes nor the newer sequencing methods used in the identification of point mutations can be routinely carried out in the laboratories of developing countries that are technology challenged, resource-poor, lack skilled personnel and have weak infrastructures and utilities.
- Community of practice. FAO and the IAEA, through their ca. half a century partnership, is investing considerable resources in training, technology transfer, strengthening of infrastructure, provision of services and in research. To achieve the greatest impacts through engagements at scale, a critical mass of partners that are engaged in similar endeavors and whose activities complement those of this partnership need to be assembled. Such a community could arise around a theme, such as climate change, for instance. Induced mutations can unmask alleles of genes that can be introgressed into breeding materials. This would clearly complement several ongoing activities by a multitude of potential partners, even on global scales, but efforts need to be invested in coalescing these efforts. The TCP and CRP models of the Joint FAO/IAEA Division of Nuclear Techniques for Food and Agriculture can be further scaled up to achieve this purpose.

Conflict of Interest

The author declares no conflict of interest. The views expressed in this publication are those of the author and do not necessarily reflect the views or policies of FAO.

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