

**INVITED REVIEW**

## MUTATIONS IN PLANT EVOLUTION, CROP DOMESTICATION AND BREEDING

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### ABSTRACT

Mutations and natural selection have been the cornerstone of plant evolution. Mutations have also played a significant role in crop domestication and plant breeding. Defined as sudden heritable changes in the DNA in living cells, not caused by genetic segregation or genetic recombination, mutations can be intragenic (point mutations), structural (chromosome rearrangements) or genome mutations (changes in chromosome number). Extranuclear or plasome mutations such as those leading to cytoplasmic male sterility are also of significance in crop breeding. Using mutation techniques, defects in elite cultivars such as susceptibility to a new pathogen can be fixed faster than backcrossing. If a gene of interest is not available in the germplasm, mutation induction could be the only non-GM pathway to introduce a new trait. Mutation breeding is the only straightforward alternative for improving seedless crops and cultivars. Since the first publications of induced mutagenesis using x-rays almost a century ago, many more potent physical and chemical mutagens have been introduced, and methodologies for using those in seed (both self- and cross-pollinated) and vegetatively propagated crops have been established. This review first looks at the role of mutations in plant and crop evolution and then describes the choices available for mutation breeding programmes in terms of available mutagenic agents, treatment methods from cell cultures to whole plants, and some of the achievements. There are exciting opportunities available for modern plant breeding through integration of mutation techniques with plant molecular approaches. More than 3400 registered mutants in over 170 species in the largest mutant database managed by FAO/IAEA is a testament to the success of practical application of the technology.

Keywords: Adaptation, Domestication, Chemical mutagenesis, Induced mutation, *In vitro* selection, Ion beam, Ionising radiation, Space mutagenesis

### INTRODUCTION

Although crop domestication commenced more than 11,000 years ago (Meyer and Purugganan 2013, Purugganan and Fuller 2009), it was in the twentieth century that targeted breeding became possible after the rediscovery of Mendel's laws at the turn of the 20th century (Fairbanks and Rytting 2001), when the science of genetics was born. Plant breeding is man-guided evolution of crop plants, and genetic variation is its foundation. The discovery of artificial induction of mutations in crop plants (Stadler 1928b) added another dimension to our ability to manipulate crop plants. A mutation is a sudden heritable change in the DNA in a living cell, not caused

by genetic segregation or genetic recombination (van Harten 1998). Mutation breeding is the purposeful application of mutations in plant breeding. When the desired variation is available in the germplasm, breeding objectives of a crop may be achieved through cross breeding. However, when parents possessing the desired traits used in cross breeding are poorly adapted, it becomes necessary to implement a back-crossing strategy to recover the elite type. Poor combining ability of parental genotypes is another problem encountered in conventional cross breeding, such as in cross breeding of aromatic rice with non-aromatic varieties (Bourgis *et al.* 2008, Pathirana *et al.* 2009). Mutation induction has a clear advantage over cross breeding under such circumstances.

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Genes conferring desirable traits are sometimes tightly linked to those for undesirable characters. Under such circumstances, mutation induction may result in a crossing over event or isolation of an independent mutation for the desired trait. Furthermore, some crop species such as edible banana, some table grape cultivars do not produce seeds. In such crops, mutation induction can help increasing variability for developing new cultivars. Root and tuber crops (Ahloowalia and Maluszynski 2001, Broertjes and van Harten 1988, Medina *et al.* 2004, Predieri 2001), ornamental plant species propagated vegetatively (Broertjes and van Harten 1988, Datta 2009, Kondo *et al.* 2009, van Harten 1998) and perennial fruit crops (Broertjes and van Harten 1988, Hensz 1978, Predieri 2001) can also benefit immensely by this method.

Since the first publication of induced plant mutations almost a century ago (Stadler 1928a, Stadler 1928b) the method has been applied actively for over a half century, particularly after the Joint FAO/IAEA Division was established in Vienna, which assisted developing countries to quickly adopt advances in the technology (Ahloowalia *et al.* 2004, Jain 2005, Pathirana 2011) and some countries with nuclear power developing their own breeding programmes in specialized institutes such as in India (Jagadeesan and Thumballi Ramabhata 2021), Pakistan (Rana 2018), Korea (Kang *et al.* 2020) and Japan (Nakagawa 2018). Widespread use of mutation technology enabled the development and release of 3365 registered mutant varieties in more than 170 different plant species to date (IAEA 2021). The number of registered cultivars according to continents is given in Table 1. Some of these mutant cultivars have revolutionized agriculture not only in densely populated developing countries but also in agriculturally advanced countries as will be evident from the sections to follow.

### Types of mutations and their role in plant evolution

Mutations can be broadly divided into (a) intragenic or point mutations occurring within a gene in the DNA sequence, (b) intergenic or structural mutations within chromosomes

**Table 1:** The number of mutant cultivars released in different continents according to the largest mutant database available (IAEA 2021)<sup>a</sup>

Continent	Number of mutant cultivars
Africa	82
Asia	2052
Australia + Pacific	9
Europe	960
Latin America	53
North America	209
Total	3365

<sup>a</sup> Not all released mutant varieties have been registered, hence the actual numbers are much higher

(inversions, translocations, duplications and deletions) and (c) mutations leading to changes in the chromosome number (polyploidy, aneuploidy and haploidy). In addition, it is important to distinguish between nuclear and extranuclear or plasmone (mainly chloroplast and mitochondrial) mutations, both being of considerable interest to agriculture. Recent molecular studies, particularly genome sequencing, show that duplications at individual gene level (Nelson and Werck-Reichhart 2011, Rensing 2014) as well as at a whole genome level (Fawcett *et al.* 2013, Vanneste *et al.* 2014) have been the cornerstones in plant evolution, although the exact timing of these events in many cases is poorly understood (Clark and Donoghue 2018). In addition to individual gene duplications that occur as tandemly arrayed paralogs or in different regions of the genome through transposition or retrotransposition, long stretches of DNA are also duplicated, and when it reaches a whole chromosome, the phenomenon is trisomy. The extreme occurrence of this duplication is at whole genome level, commonly called whole genome duplication (WGD) and is paramount to evolution as revealed by genome sequencing in members belonging to different plant families and orders, for example in Compositae (Asterales) (Barker *et al.* 2016), Brassicales (to increase chemical defense complexity against butterfly larvae) (Edger *et al.* 2015), Salicaceae (Liu *et al.* 2017),

Solanaceae (for the development of fleshy fruits as a seed dispersal mechanism), Papilionoideae (a mechanism to adapt to low nitrogen soils through rhizobial symbiosis) (Vanneste *et al.* 2014). Furthermore, grapevine genome sequencing revealed contribution by three ancestral genomes in the evolution of *Vitis vinifera*, thus making cultivated grapevine a progenitor from a hexaploid. Multiple genes coding for chemical constituents responsible for the aroma and health benefits of red grape (the sequenced plant is a highly inbred 'Pinot Noir', a red grape) such as stilbene synthases (coding for resveratrol – 43 genes) and terpene synthases (coding for many aromatic chemicals of wine – 89 functional and 27 pseudogenes as against less than 40 in rice, poplar and Arabidopsis) have been identified (Jaillon *et al.* 2007), demonstrating the multifunctional genomic machinery in an ancient crop, and the advantages of polyploidy.

Interestingly genome sequencing enabled the identification of over 10,088 genes, including many that resulted from an ancestral genome duplication event in *Amborella trichopoda* (Albert *et al.* 2013), the unique and only living representative of all other living angiosperm plant lineages. Thanks to multiple genome sequencing projects and comparative genome biology research, now we know that the ancestor of all the angiosperms on earth has had a WGD about 192 Mya and before that another WGD prior to diversification of seed plants, some 319 Mya (Jiao *et al.* 2011). Thus it is now established that all the seed plants (both angiosperms and gymnosperms) to be paleopolyploids (ancient polyploids) and that every extant angiosperm is in fact a paleopolyploid containing the remnants of at least two WGDs (Jiao *et al.* 2011). Additionally, Vekemans *et al.* (2012) describe another polyploidisation event, in fact a hexaploidy event that pre-dates the origin of all core eudicots, termed gamma triplication. Phylogenetic studies of the subfamilies of MADS-box genes showed that this occurred before the divergence of Gunnerales but after the divergence of Buxales and Trochodendrales. The authors suggest that

gamma triplication initiated the functional diversification of key regulatory elements of the core eudicots, representing 75% of flowering plants (Vekemans *et al.* 2012).

Monocots have not been spared either in multiple polyploidisation events after their emergence. They comprise nearly one fourth of all flowering plant species with the largest range of genome size variation. For example, three WGD events termed rho ( $\rho$ ), sigma ( $\sigma$ ) and tau ( $\tau$ ) have been described in the genomes of cereal grasses (Jiao *et al.* 2014, McKain *et al.* 2016). Among these WGD events  $\rho$  was the first to be described and it is estimated to have occurred ~70 Mya, before the divergence of the major cereals (Poaceae) from one another but after the divergence of the Poales from the Liliales and Zingiberales (McKain *et al.* 2016, Paterson *et al.* 2004). Jiao *et al.* (2014) demonstrated that  $\tau$  polyploidisation event is shared by all commelinids, whose origin is estimated at 120 -100 Mya, ~73 Mya before the Arecaeae-Poaceae split. Thus, many agriculturally important species such as palms, banana, ginger, cereals and grasses all have been subjected to this ancient WGD. Sigma event is estimated to have occurred prior to the diversification of Poaceae but after the divergence of Poales from other commelinid orders, ~130 Mya (Tang *et al.* 2010). Sigma event has had an enormous positive impact on the evolution of the order Poales, representing ~21,000 species encompassing ~33% of monocot species with families such as Poaceae (~11,300 species), Cyperaceae (5,700 species) and Bromeliaceae (~3100 species) (McKain *et al.* 2016) that have dominated some ecological niches. Order Zingiberales also has had similar ancient polyploidy events. For example, *Musa acuminata* has undergone three successive rounds of WGD events since its divergence from Poales (D'Hont *et al.* 2012). Palms belonging to the order Arecales and family Arecaeae have adapted to diverse environments such as deserts, saline and sandy beaches, and tropical and temperate forests, dominating some extreme tropical ecosystems. Recent whole genome sequence (WGS) assembly of dwarf coconut enabled understanding of the evolutionary

relationships within *Palmae* based on genomic variations and synteny of coding gene sequences. The results revealed that at least three rounds of genome duplication events are commonly shared by the members of *Arecaceae* family (Lantican *et al.* 2019).

The early success of land plants has thus been attributed to WGD events (Nelson and Werck-Reichhart 2011). Duplicated genes retained in genomes can serve divergent functions albeit at low rates, as long as the original ancestral copy retains its original functions (Jiao and Paterson 2014, Lynch and Conery 2000), thus enabling the species to produce 'biological innovations' for their success as highlighted in some examples above. These WGD events are regarded as macroevolutionary events allowing a whole spectrum of benefits from evading extinction risk to biological innovations. Clark and Donoghue (2018) showed that these macroevolutionary events are drivers of diversity, herbivore interactions, geographic expansions, shifts in climate niches and of lineage longevity. The two main forms of WGD, allopolyploidy and autopolyploidy will be discussed in more detail below.

### **Intragenic or gene mutations**

Gene mutations refer to single or few base pair deletions, additions or rearrangements in the DNA. Loss of one or more base pairs is referred to as a deletion and gain of a base pair/s is an insertion. If they do not occur in multiples of three nucleotides in the sequence, the result is a frame-shift mutation (within exons of coding regions). Thus a deletion or insertion of a single base pair or two may result in a significant effect, including the loss of function due to a shift within the reading code. However, the effect of such a mutation can depend also on the location where the change happens. For example, a frame-shift mutation near the 3' end of the gene will result in only a change in the terminal part of the polypeptide chain as translation takes place only in a 5'→3' direction. Under such circumstances, even a frame-shift mutation can result in a functionally similar protein.

Another type of gene mutation is base pair substitution caused often with chemical mutagens such as alkylating agents described below. This mutation is the result of incorporation of alternate bases during replication. For example, G-C base pair can be switched to A-T, C-G or T-A. As a result, one base of a triplet codon is substituted by another, resulting in a changed codon. This can result in an altered amino acid sequence resulting in a mutated protein. If a purine base is replaced by another purine base (G by A or A by G) or a pyrimidine by another pyrimidine (T by C or C by T) the substitution is called a transition. Transitions are by far the most common types of mutations and G-C to A-T transitions occur more frequently than any other base pair substitutions in nature (Ossowski *et al.* 2010) and also when induced using alkylating agents such as ethylmethane sulfonate (EMS) (Ossowski *et al.* 2010). If a purine base is substituted by a pyrimidine, or vice versa, the substitution is called a transversion. McCallum *et al.* (2000) showed that about 5% of mutations caused by alkylating agents will introduce a stop codon (nonsense mutation) in *Arabidopsis*, another 65% will be missense mutations (a codon coding for one amino acid is converted to a codon coding for another amino acid), and 30% will be silent changes, where the final protein product remains unchanged. Loss-of-function point mutations are the most common mutational lesion that is found during later stages of crop domestication (Meyer and Purugganan 2013). Thus, many mutations conferring abiotic stress tolerance such as transcriptional suppressors belonging to the C2H2 zinc finger gene family (e.g. *Zat10* in *Arabidopsis*) conferring tolerance to salinity, heat and osmotic stress (Mittler *et al.* 2006) belong to loss or gain of function mutations. Biotic stress tolerance have also been mapped to such mutations, for example resistance to powdery mildew in *Mildew Resistance Locus O (MLO)* in many plant species (Bai *et al.* 2008, Humphry *et al.* 2011). Similarly, some plant architecture (semi dwarf) mutants that were the foundation for the green revolution also belong to loss of function mutations, for example, semi-dwarf rice varieties possessing the *sd1* allele from

Dee-geo-woo-gen contain a 383-bp deletion in the *GA20ox* gene (known as *OsGA20ox2*) that introduces a stop codon (Hedden 2003), which probably results in a highly truncated, inactive enzyme. However active gibberellins are required for normal reproductive processes and Sasaki *et al.* (2002) showed the possibility that *GA20ox-1* predominantly expressed in the unopened flower enables the *sd1* rice to develop spikelet and fertilise normally not compromising the yield due to the truncated gene. It is interesting to know if these multiple alleles were the result of an ancient duplication event discussed above.

### Chromosome rearrangements or structural mutations

Chromosome breaks and their rearrangements lead to structural mutations. Ionising radiation results mostly in such changes (Kianian *et al.* 2016). Four categories of such rearrangements are: a) deletions or deficiencies, b) duplications, c) inversions and d) translocations. The majority of mutations caused by ionizing radiation are deletions (Suprasanna *et al.* 2015) and are often lethal (van Harten 1998). Nevertheless, some deletions can block biochemical pathways and, if this happens in a pathway leading to the synthesis of a toxic metabolite, such a deletion may result in a non-toxic plant product making it edible. Ionising radiation often causes DNA double strand breaks (DSB). In plants DSBs are repaired through one of two pathways: homologous recombination (HR) or non-homologous end joining (NHEJ). HR is a repair process that uses an undamaged copy of the broken region as a template to repair the break. The intact copy is usually supplied by a sister chromatid but can also be located on a homologous chromosome. Therefore, HR is generally an error-free process. In NHEJ, the ends of the single-strand chains are joined without requiring sequence homology, and extensive deletions or insertions can occur. Duplications have been a cornerstone in plant evolution (Edger *et al.* 2015, Shi *et al.* 2010, Vanneste *et al.* 2014).

An inversion is the result of an 180° rotation of blocks of nucleotides, and translocations of

these blocks to other chromosomes are also common in plant evolution. This phenomenon was first experimentally proven by Barbara McClintock (1942) in maize. She later showed that certain loci in maize are unstable and that mutations need not express changes in genes, but can be the result of changes affecting the control of genic action (McClintock 1953), which are now known as transposons. Emerging evidence of the role of chromosomal rearrangements in crop evolution suggests that crop improvement can be accelerated through the combination of distant hybridization with radiation breeding. The first example of this application was demonstrated by Sears (1956) by transferring a small section of an *Aegilops umbellulata* chromosome containing a gene for resistance to brown rust (*Puccinia triticina*) to hexaploid wheat through a bridging cross employing *Triticum dicoccoides* and irradiation.

### Genome mutations

As already mentioned, polyploid origin of all angiosperms leading to amplification of gene families and structuring of genomes is now confirmed thanks to WGS in many diverse species followed by comparative genome biology. Recurring polyploidisation followed by gene silencing, deletion etc., leading to diploidisation has been implicated in the success of land plants. Heritable increase in genome copy number (WGD), called polyploidy is a central feature of plant diversification. Additionally, loss or gain of an individual chromosome called aneuploidy is also described under polyploidy. Flowering plants have relatively large amount of DNA, genes and genome sizes due to their evolution through gene duplications, including WGD (Rensing 2014, Vanneste *et al.* 2014). Many genes duplicated as individual genes, blocks of genes in chromosomal segments or as a result of WGD undergo purifying or negative selection over time. In polyploids, this phenomenon is called diploidisation or fractionation, and this process can continue until the gene content is similar to that in the parental genome, plus the retained paralogs (Bowers *et al.* 2003, Clark and Donoghue 2018, Rensing 2014, Vanneste *et al.* 2014). Such extant species that have emerged from

polyploidisation events and subsequently behave like diploids are called paleopolyploids. (Rensing 2014, Vanneste *et al.* 2014).

Polyploidy has the advantage of improved abiotic and biotic stress tolerance enabling adaptation of species to new environments and wider distribution (Fawcett *et al.* 2013, Parisod *et al.* 2010). Polyploids possess larger seeds and fruit enabling faster establishment giving them a competitive advantage. Wood *et al.* (2009) showed that among 28,406 vascular plant species, 9,795 (34.5%) were polyploid relative to their generic base. Much progress has been achieved in understanding the consequences of polyploidy but causes of polyploidy are less well understood (Wagner *et al.* 2019). Genome mutations can be generally classified into allopolyploidy, autopolyploidy, aneuploidy and haploidy, although this classification is debatable (Parisod *et al.* 2010).

Allopolyploidy is the result of a combination of genomes of two or more species and arises through interspecific or intergeneric hybridization followed by the doubling of chromosomes. However, the more common route is through fusion of two unreduced (diploid) gametes (resulting in a tetraploid) or the fusion of a reduced gamete (haploid) with an unreduced gamete (resulting in a triploid). The latter can in turn produce autotetraploids through selfing and backcrossing. Production of unreduced gametes is a genetically programmed trait and the rates differ from one species to another. It is also accelerated by environmental stress and is a pathway for speciation and adaptation (Fakhri *et al.* 2016, Mason and Pires 2015). Lim *et al.* (2007) using Genomic In Situ Hybridisation, Tandem Repeat Characterisation and other molecular techniques demonstrated this phenomenon in *Nicotiana* allopolyploids, where deletions, insertions and homogenization of repetitive sequences resulted in a complete turnover of non-genic portions of the genome within a period of less than 5 million years, leading to structural diploidisation within a well-conserved karyotype structure. Evolution of many crops such as wheat, soybean, cotton

and *Brassica* species has taken this path (Vavilov 1992, Zhukovsky 1971). Mutant selection is often complicated by the presence of duplicated homeologous allele pairs in allopolyploids. Targeting Induced Local Lesions IN Genomes (TILLING) populations are helpful in identifying and targeting these alleles for breeding (Stephenson *et al.* 2010, Taheri *et al.* 2017, Till *et al.* 2010).

In contrast to allopolyploidy, autopolyploidy is when the chromosome number increases within a species resulting in more than two homologous chromosomes. This results in the formation of multivalents during meiosis, often leading to seed sterility in autopolyploids, particularly in the initial phase of their evolution. Although genome doubling through autotetraploidy results in immediate acquisition of novel traits such as increased gene expression, larger cell size, changes in physiological and adaptive traits, whether genome doubling itself has evolutionary advantage still remains an open question. Thus, for example, diploid cytotypes of *Santolina pectinata* Lag. (Asteraceae) in Spain show a much wider ecological spectrum than their autotetraploid counterparts (Rivero-Guerra 2008). In contrast, the diploids of *Hordeum maritimum* Hudson (sea barley) exist within the Mediterranean basin while the autotetraploids occur from Eastern Mediterranean to Asia. Jakob *et al.* (2007) showed that the polyploidy cytotype underwent pronounced ecological shift adapting to the mountainous inland habitats of Afghanistan, while still occurring in the Mediterranean basin. Analysing gene expression under drought stress, Zhou *et al.* (2019) demonstrated that the autotetraploid accessions of *H. maritimum* have more differentially expressed drought-related genes and a higher number being expressed under drought (297 and 126, respectively) compared to the diploid (186 and 97, respectively), thus enabling the autopolyploid to adapt to drought-prone mountains. Sequencing data show that rapid elimination of certain portions of DNA sequences occur after autopolyploidisation (Lynch and Conery 2000), resulting also in the reduction in chromosome length (Rivero-Guerra 2008).

Among tropical crops, edible banana was thought to have evolved at a triploid level through polyploidisation at single genome level as well as through allopolyploidy as a result of interspecific hybridization of the two wild species *Musa acuminata* and *Musa balbisiana* (Heslop-Harrison and Schwarzacher 2007). Great diversity of banana germplasm, containing starchy fruits suitable for cooking through to soft and sweet desert types, can be attributed to this divergence at a genome level. De Langhe *et al.* (2010) provide experimental data towards a hypothesis that banana evolution has occurred through backcrossing of an intermediate hybrid. Most of the modern cultivated sugarcane clones belong to *Saccharum officinarum* ( $x = 10$ ) (50% sugar content in stems on a dry weight basis) backcrossed to *S. spontaneum* ( $x = 8$ ) to transfer disease resistance traits. Comparative Genomic DNA in situ Hybridisation allows to distinguish individual chromosomes contributed by individual parents in a polyploid. Applying the method to clone 'R570' ( $2n = 107 - 115$ ) D'Hont *et al.* (1996) showed that about 10 % of chromosomes have been contributed by *S. spontaneum* with another 10% being recombinant chromosomes from both species while the rest were from *S. officinarum*. Similarly, analyzing genomic and ribosomal DNA from four Cuban sugarcane cultivars, Cuadrado *et al.* (2004) showed that these cultivars are also aneuploids (from  $2n = 102 - 106$  in 'My5514' to  $2n = 113 - 117$  in 'C236-51') and about 16% chromosomes are contributed by *S. spontaneum* and less than 5% of the chromosomes are recombinant containing sequences from both species.

Cultivated and tuber producing wild potatoes have a basic chromosome number of 12 ( $x = 12$ ) and form a polyploid series of  $2n = 2x, 3x, 4x, 5x$  and  $6x$  chromosomes (Camadro *et al.* 2012). All the landrace cultivars and wild potato species used in crop improvement programmes belong to section Petota of the genus *Solanum* with basic chromosome number of  $x = 12$ . Among 107 wild potato species Spooner *et al.* (2014) analysed, only 64 (60%) were exclusively diploid. Other

species had multiple cytotypes; diploid and triploid cytotypes, diploid and polyploid cytotypes except triploids, and another large group (18%) were exclusively polyploid at tetraploid and hexaploid levels. Additionally there were few that were exclusively triploid or pentaploid, or a mix of triploid and tetraploid cytotypes. This high variation in ploidies within the genus would have allowed the adaptation of potato from 38° N to 41° S at elevations from 2000 to 4000 m in the American continent (Spooner *et al.* 2014, Spooner and Hetterscheid 2006). Modern breeding of sugar beet is based on hybridization of diploids with tetraploids to produce hybrid triploids. Sugar beet produces the highest yield and sugar content at a triploid level (Kikindonov 2009).

Autopolyploidy can be induced by the use of chemicals that inhibit spindle fiber formation during mitosis, thus the divided chromosomes are retained in the same cell (Morgan *et al.* 2011). Homozygous lines for heterosis breeding can also be obtained using these chemicals on haploid plants produced through anther, microspore or ovule culture (Pathirana *et al.* 2011a). Haploidy (one set of chromosomes) and aneuploidy (imbalanced chromosome number) are other forms of ploidy variations. Cultivated sugarcane is a good example of aneuploidy combined with allopolyploidy as already mentioned. Perennial cereals are much environmentally friendly and attempts are being made to convert major cereal crops perennial. Fu *et al.* (2015) demonstrated a pathway for whole chromosome introgression of cultivated maize (*Zea mays*  $2n=20$ ) with a distant perennial relative *Z. perennis* ( $2n=40$ ), the only tetraploid member of the genus *Zea* through aneuploidy to overcome the barriers of chromosome introgression.

#### **Extranuclear or plasmone mutations**

Higher plants have the unique feature of having two extra-nuclear genomes, in the chloroplasts and mitochondria. Mutations in the genomes of these organelles can be of value in crop improvement. These mutations occur spontaneously and can also be induced using mutagens or through plastid

transformation. Inheritance of plastid mutations are carried through the cytoplasm of the maternal parent, and hence do not behave in a Mendelian fashion. These mutations have been used in breeding horticultural crops with various leaf or fruit characters as they often occur as chimeras and passed on through vegetative propagation. Fungal disease resistance, herbicide tolerance and cytoplasmic male sterility are some of the important characters often inherited through cytoplasmic genes, although complex nuclear cytoplasm interaction in the expression of these characters have been reported (Clements *et al.* 2012, Elkonin 2005).

### **Mutagenic agents and their use in mutation breeding**

The agents used to induce mutations are called mutagens. These can be either chemical or physical. In principle, any propagule capable of producing a new organism can be used as the object to induce artificial mutations. As plants have totipotency, i.e. capability of giving rise to a new plant from a single living cell, this propagule can be a single cell (such as in a well-dispersed cell culture). Thus mutagens have been used on the whole spectrum from cell and tissue cultures (Elkonin *et al.* 2010, Elkonin and Tsvetova 2008, Fuchs *et al.* 2005, Patade *et al.* 2008, Pathirana *et al.* 2002, Penna *et al.* 2008, Saito *et al.* 2001) to whole plants, particularly in gamma fields and gamma greenhouses (Ahmad *et al.* 2018, Kang *et al.* 2010). However, for seed propagated species, the vast majority of mutation breeding programmes have used seed treatment as evident from the list of released mutant cultivars and their origin (IAEA 2021).

### **Physical mutagens**

Ionising radiation, mainly from X-rays was exclusively used in the early period of mutation research until the effects of certain chemicals on the DNA were published in the mid-1940s (Auerbach and Robson 1946, Rapoport 1946). Radiation from  $\gamma$ -rays from radioactive elements such as  $^{60}\text{Co}$  and  $^{137}\text{Cs}$  became popular as they were made available to many developing countries through the IAEA. Before the introduction of chemical

mutagenesis, this approach was called radiation breeding. Radiation is described as energy (travels in the form of waves) or subatomic particles that travel through space or other medium. Radiation can be corpuscular (travels in the form of subatomic particles) or electromagnetic. Both X-rays and  $\gamma$ -rays belong to non-particulate ionising radiation. Fast neutrons are another source of ionising radiation used in plant mutagenesis, and are emitted from nuclear reactors. This irradiation source is available for use in mutation research through the Joint FAO/IAEA Division in Vienna. Additionally, several countries having their own nuclear reactors, have made these facilities available for plant breeders. In addition to acute irradiation (high dose during short period), chronic irradiation facilities have also been developed, where plants are allowed to grow in the presence of low-dose ionizing radiation, either under field conditions (gamma field) or in a greenhouse (gamma greenhouse). Traits targeted in 22 species in a gamma greenhouse established in 2009 in Malaysia and the results have been summarized by Ahmad *et al.* (2018). Using chrysanthemum as an example, Nagatomi and Degi (2009) showed that a larger variation in petal colour can be achieved using chronic irradiation on callus cultures compared to acute irradiation, and that the radiation damage can be overcome in proliferating callus when chronic radiation is used. Importantly, the combination of callus culture and chronic irradiation produced a mutation rate eight times higher than the conventional acute irradiation of cuttings, in addition to producing non-chimeric mutants (Nagatomi and Degi 2009, Yamaguchi *et al.* 2008).

Ion beam radiation is another source of physical mutagen used successfully in recent years, particularly in Japan using high-energy (220 MeV) (Ryuto *et al.* 2008, Tanaka *et al.* 2010, Yamaguchi 2018) and in China with low energy (30 KeV) (Wang *et al.* 2005, Ya *et al.* 2011, Zhou *et al.* 2006). They are usually generated in particle accelerators and have a mass and an electrical charge. Therefore linear energy transfer (LET) in ion beams is different to gamma- and X-rays



which have neither a mass nor an electrical charge. High-LET radiation, such as ion beams, causes more localized, dense ionization within cells than low-LET radiation. Again, not all the types of ion beams are equally effective in mutation induction. Resistance to bacterial leaf blight and blast in rice (Xiao *et al.* 2008), yellow mosaic virus in barley (Tanaka *et al.* 2010), potato virus Y in tobacco (Hamada *et al.* 1999) and black sigatoka disease in banana (Reyes-Borja *et al.* 2007) are a few examples of useful mutants developed through ion beam irradiation. Many ornamental crop cultivars with unique colour characteristics have been developed as well (Magori *et al.* 2010, Tanaka *et al.* 2010). Tanaka *et al.* (2010) recently summarized the results of 19 years of work in different crops and concluded that the spectrum of mutations induced by ion beam radiation is different from that of gamma rays, and novel mutant types hitherto not produced have been isolated in ornamental plants. However, in rice the spectrum of different ion beams (C and He) was similar to that of gamma rays, but frequency was higher (Yamaguchi *et al.* 2009a).

In chrysanthemum for example, 220 MeV carbon ion beam produced higher mutation rates with lower DNA damage compared to 320 MeV carbon ions, 100 MeV helium ions and gamma rays (Yamaguchi *et al.* 2010). Furthermore, studying sectoral chimeras produced after irradiation, Yamaguchi *et al.* (2009b) found that regenerative capacity of mutated sector is greater in ion beam radiated shoots compared to gamma-ray treated shoots with the possibility of recovery of solid mutants in the former. Higher mutagenic efficiency of ion beams over gamma rays was also found in rice, but there were no differences in efficiency of mutation induction in the types of ion beams investigated (Yamaguchi *et al.* 2009a). Using an interspecific hybrid of cyclamen producing purple flowers due to the accumulation of malvidin 3,5-diglucoside, Kondo *et al.* (2009) were able to isolate a red-purple mutant after irradiating the etiolated petioles of the hybrid with carbon ion beams at 320 MeV and 16

Gy. This mutant was found to accumulate delphinidin 3,5-diglucoside due to the loss of methylation of anthocyanin 3'- and 5'-hydroxyl groups. This enabled the researchers to add delphinidin type to the flower colour range in cyclamen, which was previously restricted to malvidin, peonidin and cyanidine types (Kondo *et al.* 2009). Additional information on the use of ion beam mutagenesis can be found in a review by Magori *et al.* (2010) and for ornamental crops by Yamaguchi (2018). Table 2 describes some of the properties in the electromagnetic radiation spectrum and corpuscular radiation including those with mutagenic and ionising effects.

In addition to the radiation options listed in Table 2, space radiation (cosmic rays) has also been used for mutation studies and for crop breeding in recent years (Gao *et al.* 2009, Li *et al.* 2020, Ou *et al.* 2010). It was postulated that the environment of the space flight has more benefits for mutagenizing biological material than on earth because of the interaction of microgravity with the mutagenic process created by the cosmic rays (Guo *et al.* 2010, Hu *et al.* 2010, Ou *et al.* 2010). The data from DNA analyses of rice plants from seeds irradiated in satellites have confirmed high rates of epigenetic changes and transposon activation (Guo *et al.* 2010, Hu *et al.* 2010, Long *et al.* 2009, Ou *et al.* 2010). Comparing the spectrum of epigenetic changes after space flight with those after two ion beams differing in LET and dose, Shi *et al.* (2014) found that 50% of the mutation sites were common in the two ion beam-irradiated populations, while only 20% were common between space-irradiated and ion beam-irradiated populations. Furthermore, both ion beams and space induced mutations were widely spread across the genome covering coding and repeated regions. However, methylation changes were more in CNG sequence in the case of space flight whereas CG sequence was affected more by ion beam radiation (Shi *et al.* 2014).

Spacecraft, recoverable satellites and high altitude balloons have been used and more than 60 cultivars have been released as a

Table 2. Electromagnetic and particulate radiation, their sources and biological effects (from multiple sources)

Type of radiation/ electromagnetic (EM) wave	Source	Type	Biological and other effects	Details
Extremely low-frequency	Power plants, high voltage cables, electrical wiring and household electrical equipment, thunderstorms, lightning	Non-ionising, electromagnetic	Inconclusive leukemia, cancer link	Low-energy radiation often referred to as low frequency, part of radio waves in the electromagnetic spectrum
Radio waves	Coil transmitter, astronomical objects with changing magnetic fields, lightning, radio (AM/FM), mobile phones, Wi-Fi	Non-ionising, electromagnetic	No conclusive human health effects on long-term exposure	Part of electromagnetic radiation, has long wavelengths in electromagnetic spectrum
Microwave radiation	Magnetron, radar transmitters, FM radio antennas	Non-ionising, electromagnetic	Energy loss high with distance, therefore safe. Heat injury possible	Used in communication, radar, police speed guns, GPS and cooking. 1-300 GHz frequency and 1 mm – 30 cm wavelength. Comes in different bands for a variety of applications
Infrared radiation	Solar radiation, high pressure sodium lamps, heating devices, infrared lamps	Non-ionising, electromagnetic	Heating in greenhouses, better plant growth at low ambient temperatures	760 nm and 1,000,000 nm wavelength, therapeutic uses – neural stimulation, wound healing, cancer treatment
Visible light	Solar radiation, light, fluorescent and LED bulbs	Non-ionising, electromagnetic	400 - 500 nm blue can cause retinal injury	400 – 750 nm wavelength, photosynthetically active radiation (400 – 700 nm).
Ultra violet light (UV)	Solar radiation, mercury vapor lamps, tanning beds, sparks, arcs	Both ionizing and non-ionizing electromagnetic <sup>a</sup>	UVC most damaging to skin. Induces purine and pyrimidine dimers, mutagenic	100 – 400 nm wavelength UVA (315-400 nm) UVB (280-315 nm) UVC (100-280 nm) Low energy and poor cell penetration, can use on pollen and callus cultures for mutation induction
$\alpha$ radiation (Alpha radiation)	$\alpha$ particles are produced during radioactive decay of naturally occurring U, Th, Ra and man-made elements such as Pu, Am, Po-210.	Particulate ionising radiation, consists of two protons and two neutrons (He nucleus)	Can't penetrate skin, but can be lethal if ingested. High ionising power, low penetration. Mutagenic	Strong interaction with matter but travels few cm in air. Penetration of plant tissue poor – less than 400 $\mu$ m
$\beta$ radiation (Beta radiation)	$\beta$ decay of naturally occurring radioactive elements (U, Th, Ac), S-35, C-14 and products of nuclear fission Sr-90, Cs-137	Particulate, ionising. Fast electrons and positrons emitted from an atomic nucleus during radioactive decay	Can penetrate skin, but health risk is by ingestion. Mutagenic	Electron or positron, travels few meters in air, can't penetrate few mm plastic. Can penetrate few cm in plant tissue

Continued table 2;

Type of Radiation/ electromagnetic (EM) wave	Source	Type	Biological and other effects	Detail
X-rays	Originates from the electron cloud of an atom. X-ray machines (e.g. CT scanner)	Ionising electromagnetic radiation	DNA and chromosome damage	High energy, widely used in mutation breeding (seeds, plant parts), deep penetration of tissue
$\gamma$ -rays (Gamma rays)	Electromagnetic radiation emitted by the nucleus of radionuclides during radioactive decay. Emitted during natural decay of U, Th, Ac and from naturally occurring K-40 and C-14. Artificial $\gamma$ emitters such as Co-60, Cs-137, Am-241 are used in science, industrial and medical applications	Ionising electromagnetic radiation	DNA and chromosome damage	More energetic and penetrative than X-rays (several MeV), widely used in mutation breeding (seeds, plant parts)
Neutrons (slow, fast and thermal)	Neutrons are produced by neutron sources (e.g. Cf-98), nuclear fission and fusion reactions, and particle accelerators	Neutron particle; fast neutrons have high energy (1 – 20 MeV) and velocity ( $2 \times 10^4$ km/s)	Travels long distance, stops by water or concrete. DNA damage	Indirectly ionizing (cause ionizing radiation) (neutral) but its high energy makes other objects radioactive. Used in mutation breeding, can penetrate several cm in plant tissue
Ion beams	Particle accelerators (Cyclotron)	Ionised nuclei, e.g. $^{12}\text{C}^{6+}$ , $^{14}\text{N}^{7+}$ , $^{56}\text{Fe}^{24+}$ , $^{34}\text{Ar}^{18+}$	Very high energy, DNA damage	Few cm penetration in plant tissue, used in the region 100 – 320 MeV for mutagenesis

<sup>a</sup>The ionization energy of molecules and atoms (10 eV to about 33 eV) falls in the UV range. The low energy portion of the UV below the ionization energies is non-ionizing while at higher energies, UV is ionizing

result of this programme. Molecular and segregation analysis of three blast-resistant rice mutants induced by space flight revealed that one or two dominant mutations were responsible for resistance to different isolates of the pathogen (Xiao *et al.* 2008). Discovery of dominant mutations from space flight is noteworthy and may be indicative of a different spectrum from other mutagenic agents.

Lotus (*Nelumbo nucifera* Gaertn) seeds and rhizomes have medicinal properties and also used as food in Asia. A lotus variety named 'No 36' developed after seed irradiation in a recoverable satellite has been grown for six years in Fujian Province, China. Comparison of seeds of 'No. 36' with native parent showed higher protein and phospholipid content as well as 100 seed weight in 'No. 36' (Wu *et al.* 2007). Facilities for life science research including cosmic irradiation

facilities on board recoverable science satellite SJ 10 have been described by Zhang *et al.* (2019). Work undertaken in the USA has finally resulted in sending a large consignment of grapevine cultivars on board International Space Station in early 2021 with the plan of inducing epigenetic variation for adaptation to extreme climates on earth, in addition to jute and coffee tissue cultures that were sent to space in 2020 (Wired 2021).

Ionising radiation results in the breakage of hydrogen bonds, sugar phosphate moiety and chromosomal breakages allowing cross-linking of DNA strands. Among physical mutagens, ultra violet (UV) rays have low penetration and are effective in producing purine or pyrimidine dimers resulting in point mutations. UV rays can be effectively used to irradiate pollen in the late or early uninucleate stages (Toker *et al.* 2007, van Harten 1998). Radiation doses and conditions for irradiating

seeds and buds, planning of radiation experiments, handling and selecting in mutant populations have been published (IAEA 1977, IAEA 2011, Mba *et al.* 2010, Medina *et al.* 2004, Predieri 2001, van Harten 1998), and therefore will not be enumerated here. Treatment conditions and doses need to be experimentally derived for new or minor crop species as information may not be readily available. A suitable dose for irradiation has often been recommended as the one that reduces growth and morphogenic performance to 50% (Growth Reduction - GR 50) of untreated controls (IAEA 1977, IAEA 2011, Novak *et al.* 1990), but for seeds it is recommended to use 2–3 doses (IAEA 1977). The main external factors affecting irradiation are oxygen and moisture contents, temperature and post-irradiation storage conditions (IAEA 1977, van Harten 1998).

### **Chemical mutagens**

Ionising radiation is available for plant scientists at highly regulated installations with radiation levels monitored and all necessary precautions for safety already in place. In contrast, chemical mutagens are available for purchase and often breeders set up their own experiments in their own lab settings. All mutagenic agents are carcinogenic and toxic, therefore it is important to use them with care, with precautions taken not to inhale, spillage etc. and with proper disposal procedures in place. For example, it is not recommended to treat plant material in standard horizontal laminar hoods which have no protection for the worker. On the other hand, *Class II* biosafety cabinets offer vertical airflow but if not ducted to outside, still there is a chance of compromising personal safety. Therefore it is ideal to use *Class II Type B* biosafety cabinets that are hard-ducted to outside. These can be found in many chemistry and biochemistry laboratories. Material Safety Data Sheets available with suppliers or in the internet should be consulted for safe use.

Search for alternatives to radiation for mutation induction resulted in the discovery of an array of potent mutagenic chemicals. The different actions on the DNA, penetrative capacity, and solubility of the wide variety of

chemical mutagens make generalization of treatment conditions difficult. This is further complicated by the different plant materials used, such as seeds, pollen, growing shoots, *in vitro* cultures etc. that have different levels of mutagen uptake. Nevertheless, in practical plant mutation breeding, only a limited number of chemical mutagens in the group of alkylating agents have been used in developing absolute majority of the known mutant cultivars, for example, among those registered in the IAEA mutant database (IAEA 2021).

Chemical mutagens can be broadly divided into alkylating agents (AAs), base analogs, intercalating agents, topoisomerases, nitrous oxide and antibiotics. AAs are the most popular for inducing intragenic mutations, and among those, EMS stands out due to its effectiveness and ease of handling, especially its detoxification through hydrolysis for disposal. According to the number of alkyl groups in the molecule, AAs are classified as mono-, bi- or multi-functional. Other widely used alkylating agents are 1-ethyl-1-nitrosourea (ENU), 1-methyl-1-nitrosourea (MNU), diethyl sulfate (DES) and dimethyl sulfate (DMS). Nitroso compounds are light-sensitive and more precautions need to be taken due to their higher volatility than EMS. Nitrogen mustard was one of the first alkylating agents used in plant mutagenesis, and Auerbach (1949) summarized the results of chemical mutagenesis until the end of 1940s. Since then more potent and less cytotoxic mutagenic chemicals have been discovered. Their use for mutation breeding of seed and vegetatively propagated crops have been described with some protocols in literature (IAEA 1977, IAEA 2011, Mba *et al.* 2010, Medina *et al.* 2004, Predieri 2001, van Harten 1998) and therefore will not be elaborated in this review.

### **Other approaches for mutation induction**

Since a large proportion of basic research on plant mutations has been accomplished for temperate cereals, most of the straightforward recommendations relate to them. However, the methods have been successfully adapted to rice and other tropical cereals,

legumes, oilseeds and vegetables as demonstrated by the growing numbers of released cultivars – >3400 registered in over 170 species (IAEA 2021).

Fast development of *in vitro*-based methods in the past two decades has helped plant breeders to develop more efficient mutation induction and screening processes using plant cell and tissue cultures. Chemical mutagens are more popular in *in vitro* mutation induction, although irradiation can also be applied at low doses (Jain 2006, Medina *et al.* 2004, Patade and Penna 2009, Patade and Suprasanna 2008, Pathirana *et al.* 2002, Predieri 2001). This has been particularly useful in developing novel variation in vegetatively propagated crops such as banana (Ahloowalia 1997, Chai *et al.* 2004, Dizon *et al.* 2007, Pestanana *et al.* 2011, Roux *et al.* 2009), cassava (Jain 2005), sweet potato (Ahloowalia 1997, Ahloowalia and Maluszynski 2001), ulluco (Pathirana *et al.* 2011b), sugarcane (Kenganal *et al.* 2008, Patade and Penna 2009, Patade and Suprasanna 2008, Patade *et al.* 2008, Suprasanna *et al.* 2009), *Citrus* sp. (Somsri *et al.* 2008), Indiangrass (Stephens 2009), kiwifruit (Pathirana *et al.* 2016) and many ornamental species (Ahloowalia 1997, Jain 2006, Medina *et al.* 2004). *In vitro* cultures have the advantage of producing large mutant populations in a limited space, and the possibility of screening for some useful characters such as tolerance to salt (Kenganal *et al.* 2008, Suprasanna *et al.* 2009), toxic elements (Pathirana *et al.* 2002) or fungal toxins (Pathirana *et al.* 2008) in the laboratory environment. This allows the transfer of only putative mutants for field screening, and in the case of vegetatively propagated crops, removal of chimera through several subcultures. *In vitro* mutation induction and screening methods have been established for widely cultivated crops such as banana, potato, cassava, sweet potato and sugarcane (Ahloowalia 1997, Broertjes and van Harten 1988, Chai *et al.* 2004, Hamada *et al.* 1999, He *et al.* 2009, Jain 2005, Jain 2006, Medina *et al.* 2004, Novak *et al.* 1990, Patade and Suprasanna 2008, Predieri 2001, Roux *et al.* 2009).

Insertional mutagenesis is a useful method for producing mutants to investigate gene function because those mutations are tagged with DNA fragments of known sequence. There are two methods to construct such mutants: T-DNA insertions and transposon tagging. T-DNA insertions are considered genetically modified, carrying T-DNA sequence from *Agrobacterium tumefaciens* (Vain *et al.* 2008) and thus pose difficulties associated with regulatory barriers to GM crops in an agricultural environment. On the other hand, transposable elements are an internal genetic factor that has been harnessed recently for mutation breeding through their transcriptional activation. These elements can get incorporated into various regions of the plant genome (insertion mutations) and their position can be identified by PCR-based methods or *in silico* screens of databases that contain the DNA sequences flanking all insertions (Kuromori *et al.* 2009, Miyao *et al.* 2007, Waugh *et al.* 2006). Transgenic copies of the maize Activator/Dissociation (Ac/Ds) transposon system has become a very popular tool for gene tagging and functional genomics in various plant species and thus for “targeted mutations”. Populations carrying such insertional mutations have been developed not only for the model plants *Arabidopsis* (Kuromori and Shinozaki 2009, Myouga *et al.* 2010) and *Lotus japonicus* (Horst *et al.* 2007), but also for economically important food crops such as rice (Jiang *et al.* 2007, Miyao *et al.* 2007), barley (Ayliffe and Pryor 2009), maize (Fernandes 2004, Yi *et al.* 2009) and soybean (Mathieu *et al.* 2009). High density of mutations in these populations has helped researchers to move beyond functional genomics and use them in crop improvement programmes (Jiang *et al.* 2007, Jiang and Ramachandran 2010). Jiang *et al.* 2007 and Jiang and Ramachandran (2010) have reviewed the different mutant rice populations available for genetic analysis and estimated that the rice genome encodes about 32,000 protein coding genes.

Restriction endonucleases have been successfully used to induce double strand breaks resulting in mutations (Stoilov and Gecheff 2009, Stoilov *et al.* 1996). Further

perfection of technology has now seen the use of zinc finger nucleases (zinc finger DNA binding proteins) for inducing site specific mutations (Cai *et al.* 2009, Osakabe *et al.* 2010).

### Objectives in mutation breeding and outcomes

In the early period of mutation research it was considered that use of mutagenic agents can merely increase the rate of natural mutations. With the accumulated data, now it is clear that the spectrum of mutations generated by different mutagens is not the same. Therefore breeders can plan using particular mutagens depending on the targeted trait. This review will not attempt to describe the methodology of mutation breeding as it is well documented.

### Expanding geographic adaptation

As discussed earlier, mutations have played a key role in plant evolution, crop domestication and adaptation. Crop yield increases attributed to changed plant architecture to suit modern agronomic practices such as dwarfing in cereals (Kharkwal and Shu 2009, Rutger 2006, Rutger 2009, Rutger and Bryant 2004), bushy and determinate flowering mutants of legumes (D'Souza *et al.* 2009, Gaur *et al.* 2008, Haq 2009, Muangprom *et al.* 2005),

compact architecture in cotton (Ahloowalia *et al.* 2004, Haq 2009), sunflower (Ahloowalia *et al.* 2004, Jambhulkar and Shitre 2009, Soldatov and Kalaidzhan 1991) and castor (Ashri 1982) are also the result of inducing and incorporating mutant genes into old cultivars. Additionally, mutations conferring insensitivity to photoperiod allow their cultivation in any season such as 'PKOS1' selected from the photoperiod responsive Thai jasmine rice cv 'KDML 105' after seed irradiation with ion beams (Phanchaisri *et al.* 2007). Few examples of adaptive traits developed in rice and barley are given in Table 3.

Natural mutations and selection by man has been the driving force in the evolution of crop plants (Fisher 1999, Vavilov 1992). Barley is an excellent example of geographic adaptation using induced mutagenesis. Along with maize (Stadler 1928a), it was the first plant species subjected to induced mutagenesis by Stadler (1928b), just one year after the first artificially induced mutations were reported in *Drosophila melanogaster* by Muller (1927). Barley became a model plant for mutagenesis research and by 1988 over 1200 early maturing barley mutants were isolated in Sweden alone (Zakhrabekova *et al.* 2012). Lodging resistant mutant Mari with

**Table 3:** Some examples of adaptive traits in rice and barley developed through mutation induction

Species, Cultivar	Mutant line, Trait	Mutagen	Detail	Reference
<i>Oryza sativa</i> ssp. <i>indica</i> , 'KDML 105'	1) PKOS1, 2) TKOS4, 3) BKOS6	(1) 60, (2) 80 and (3) 60 kV nitrogen ion	All photoperiod insensi- tive; 1 & 3 Short stature; 2 & 3 early flowering	Phanchaisri <i>et al.</i> (2007)
<i>Oryza sativa</i> ssp. <i>indica</i> , 'N22'	'NH 219', drought tolerance	EMS, selected in M3	High pollen viability and spikelet fertility.	(Panigrahy <i>et al.</i> 2011, Poli <i>et al.</i> 2013)
<i>Oryza sativa</i> ssp. <i>japonica</i> , 'Calrose'	'Calrose 76', Semi dwarf, 25 cm shorter than Calrose	250 Gy <sup>60</sup> Co gam- ma-rays	<i>Sd1</i> is allelic to DGWG 'green revolution' rice, single recessive. Served as parent to 25 other culti- vars in California (13), Egypt (2) and Australia (10)	Rutger <i>et al.</i> (1976); Rutger (2009)
<i>Hordeum vulgare</i> , 'Bonus'	Mari; Short stature, earliness (8-21 days earlier than parent), lodging resistant and high yield across Scan- dinavia	X-rays, Isolated in 1950 from M2 pop- ulation and named 'early-a' or 'mat- a.8',	Released commercially in 1961. It's daughter culti- var 'Mona' is adapted to conditions in Equator (Columbia)	Dormling <i>et al.</i> (1966) (Zakhrabekova <i>et al.</i> 2012)

almost 3-week earlier heading allowed the extension of barley cultivation to northern Scandinavia and Iceland, and remarkably its insensitivity to photoperiod in the background of daughter cultivar ‘Mona’ produced high yields even under tropical conditions of Cambodia (Sigurbjörnsson 1975). Zakhrebekova *et al.* (2012) identified homology of mutant alleles *Praematurum-a* (*Mat-a*) leading to early heading in barley to *Arabidopsis thaliana* circadian clock regulator *Early Flowering 3* (*Elf3*). They characterized 87 different early heading barley mutants and found over 20 different *Mat-a* alleles with distinct mutations leading to a defective ELF3 protein. Göransson *et al.* (2021) using genome-wide association study found that this locus is also responsible for maturity date along with another three loci. Thus, modern DNA technology enables identifying the genes and mechanisms underlying the changes in these mutants.

#### **Abiotic stress tolerance and adaptability traits**

About 12% of the planet’s ice-free land surface (18 million km<sup>2</sup>) is currently cropped but a large part of the remaining land surface is either forested or unsuitable for agriculture (Ramankutty *et al.* 2002). Increasing biotic and abiotic stresses under climate change is felt across the farmlands and orchards in all parts of the world. Hence, resilient crops are needed that can tolerate increasingly changing climate and soil conditions such as droughts, temperature fluctuations, salinity, water logging and ion toxicities. Mutants with tolerance to abiotic stresses such as salinity, acid soils, drought and toxic soil conditions have enabled breeders to develop crop cultivars adapted to adverse soil environments. Adaptability traits such as photoperiod (Abdurakhmonov *et al.* 2007) and temperature response (Panigrahy *et al.* 2011), changes in breeding systems of crops affecting cross- or self-incompatibility and male sterility (Elkonin 2005, van Harten 1998) are among many other characters that have been changed to produce new cultivars through mutation breeding. The gamma ray-induced ‘Zhefu 802’ rice cultivar with a shorter growing season, tolerance to cold and

rice blast disease and high yield potential under low input conditions was the most widely planted variety in China in 1986–1994. During this period its cumulative planted area reached 10.6 million ha (Kharkwal and Shu 2009). A concerted effort in mutation breeding of rice in Vietnam has resulted in a total area of 2.54 million ha of mutant varieties cultivated in the period 2000–08 in the Mekong Delta Region, with eight rice mutant varieties adding an estimated return of US\$374 million. These mutant varieties have early maturity allowing cultivation of three rice crops annually, lodging resistance, acid sulphate soil tolerance and resistance to major pests requiring two- to three-fold less pesticide sprays during the growing period (Do 2009, Do *et al.* 2006, Tran *et al.* 2006, Vinh *et al.* 2009). A total of 32 rice varieties have been released in Vietnam alone and the ‘DT10’ variety had reached a cultivation area of 1 million ha in the period 1990 – 2009 (Khanh *et al.* 2021). Similar successes with mutant varieties of rice have been reported from Australia, Thailand, Japan, India, Costa Rica and Myanmar (Ahloowalia *et al.* 2004, Kharkwal and Shu 2009, Nakagawa 2009). Four heat tolerant rice mutants selected in the third generation after EMS treatment of seeds of highland rice variety ‘Nagina 22’ have been shown to be higher in chlorophyll content and photosynthetic efficiency than parent variety and accumulate less reactive oxygen species under heat stress, thus enabling them to stay green under heat stress (Panigrahy *et al.* 2011). Leaf proteome analysis of ‘NH219’ rice mutant using two dimensional electrophoresis, Poli *et al.* (2013) showed constitutive expression of ribulose biphosphate carboxylase large chain precursor (EC.4.1.1.39) in the mutant, and also the mutant showed differential expression above two-fold in all the 54 spots analysed.

As the Indica type semi-dwarf donors did not have an acceptable grain quality, rice breeders in California successfully used induced mutations to produce semi-dwarf mutants in Japonica and Javonica backgrounds; ‘Calrose 76’ was the first direct release semi-dwarf cultivar produced through seed irradiation with gamma rays, released in 1976 (Table 3).

Already in the early 1980s the added value to rice farmers in California from the induced semi-dwarf mutant was US\$ 20 million per year due to a yield advantage of 14% over the tall Japonica types (Rutger 2006, Rutger 2009). Genetic evaluation showed that the semi-dwarfing gene in ‘Calrose 76’ was allelic to *sd1* in the indica “green revolution” varieties derived from Dee Geo Woo Gen (Foster and Rutger 1978). Cross-breeding has resulted in 25 semi-dwarf varieties bred and released in the USA, Australia and Egypt that trace their ancestry to ‘Calrose 76’ (Rutger 2009). Mutation breeding has also contributed other semi-dwarf mutants non-allelic to *sd1*; waxy endosperm, early maturity and Basmati-type aromatic semi-dwarf mutants to the California breeding programme (Rutger 2006, Rutger 2009).

In cotton, a mutant cultivar ‘NIAB 78’ released for cultivation in Pakistan with high adaptability traits and better fiber quality recorded an additional cumulative income of US\$612.4 million from 1983 to 2005 (Haq 2009). Although the economic analyses have not been published, sunflower became an intensive crop as a result of the many dwarf mutants developed in the early years of its cultivation (Soldatov and Kalaidzhan 1991). Radiation breeding is one way of adapting crops to new environments. When cross-breeding is not an option such as in the case of aromatic rice, mutation induction and screening in the new environment may enable quick adaptation of the crop (Pathirana *et al.* 2009, Rutger 2006, Rutger and Bryant 2004).

### **Biotic stress resistance**

Development of resistant mutants have saved some crops from diseases that had been devastating for growers. Powdery mildew resistance in barley was one of the first examples. At least 32 independent mutations in the *mlo* locus have been reported and, of these, most have been induced by chemical mutagens, five with radiation and one (*mlo11*) occurs naturally (Mejlhede *et al.* 2006). Durable resistance to Verticillium-wilt in peppermint, a sterile allohexaploid (*Mentha x piperita*), was induced in 1959 by irradiation, and the resistance of newly bred cultivars was

still effective after 30 years (Micke 1993, Murray and Todd 1972, Todd 1990). Resistance to black spot disease (*Alternaria alternata* pv. Japanese pear), considered to be the most serious disease in Japanese pear (*Pyrus serotina* var. *culta*), was induced in Japan in the 1960s through chronic irradiation of the cultivar ‘Nijisseiki’ and a resistant cultivar ‘Gold- Nijisseiki’ was released (Ahloowalia *et al.* 2004, Sanada *et al.* 1988). The success of this programme led to radiation breeding of susceptible ‘Shinsui’ and ‘Osa-Nijisseiki’ cultivars resulting in resistant cultivars ‘Kotobuki-Shinsui’ and ‘Osa-Gold’ (Ahloowalia *et al.* 2004, Masuda *et al.* 1994, Nakagawa 2009, Predieri 2001). The ‘Osa-Gold’ mutant has the added advantage of being self-compatible, eliminating the need to grow pollinators (Ahloowalia *et al.* 2004). The development and introduction of phytophthora-resistant sesame mutants through radiation breeding resulted in stabilizing the declining sesame yields in Sri Lanka (Pathirana 1992, Pathirana *et al.* 2000).

There are many other examples of disease-resistant mutants developed either through direct selection after mutation induction or through cross-breeding with mutants (IAEA 2021, van Harten 1998). A good example of induced virus resistance is the cultivar ‘NIAB Karishma’, a cotton hybrid of the mutant cultivar ‘NIAB 86’, released in 1996 and resistant to cotton leaf curl virus (IAEA 2021). During the period 1997–2005, it brought additional income amounting to US\$ 294.4 million to farmers in Pakistan (Haq 2009). A number of pulse crop mutants with disease resistance have contributed to sustainable farming in India. Among these, bacterial pustule resistance in ‘TAMS 38’ soybean; powdery mildew resistance in mungbean mutants ‘TARM 1’, ‘TARM 2’, ‘TARM 18’ and ‘TM 96-2’; yellow mosaic virus resistance in mungbean mutants ‘TMB 37’ and ‘TJM 3’; and yellow mosaic virus resistance in urdbean mutant ‘TU 94-2’ are noteworthy (D’Souza *et al.* 2009). Combination of targeted mutation breeding techniques including the use of TILLING populations (Piron *et al.* 2010) or HRC and



the recently proposed strategy of disabling plant disease susceptibility genes (S-genes) (Pavan *et al.* 2010) will result in many more durably resistant cultivars developed through mutation induction.

### **Development of new crops, improvement of crop quality and nutritional traits**

Changing the product quality through mutation breeding resulted either in new products from existing crops, such as the development of a new edible oil linola (Dribnenki and Green 1995, Dribnenki *et al.* 1996, Dribnenki *et al.* 2003, Dribnenki *et al.* 2004, Dribnenki *et al.* 2005, Dribnenki *et al.* 1999, Dribnenki *et al.* 2007) utilizing two induced mutants of flax isolated in Australia (Green 1986, Green and Marshall 1984), or the overall improvement of product quality that resulted in the widespread use of a crop, such as in the case of a high oleic acid sunflower mutant that was bred in Russia (Soldatov 1976) and used extensively in the breeding programmes in North America and Europe to produce high oleic acid sunflower hybrids (Ahloowalia *et al.* 2004, Fick and Miller 1997). The reduction or complete removal of toxic substances such as alkaloids in lupines (Micke and Swiecicky 1988) and glucosides in *Melilotus* species (Micke 1962, Scheibe and Micke 1967) helped the introduction of these crops into agriculture.

The success in the rice mutation breeding programme in Vietnam encouraged the researchers to expand the work to integrate several quality traits through combining mutagenesis with hybridisation. Tran and Ho (2017) describe a protocol that the Vietnam Department of Agriculture and Rural Development uses to incorporate aroma and high iron traits and this protocol is simple and can be adopted for pyramiding other mutant traits as well.

Oilseed meals low in phytic acid is desirable in poultry and swine feed and is also environmentally friendly by reducing phosphate loads to agricultural lands and ground water. An EMS-induced low phytate mutation in a non-commercial genotype of soybean has been transferred to a commercial

cultivar through a marker assisted back-crossing programme (Landau-Ellis and Pantalone 2009). Similarly, the two-row barley cultivar 'Clearwater' has low phytate (40–50%) and derives from 'Pmut640', a sodium-azide generated mutant. Inorganic P content in the seed has increased by 400% as a result (Bregitzer *et al.* 2008). Studies have shown that these changes are associated with increased feed quality and reduced environmental impacts when fed to non-ruminant animals, based on increased P digestibility and reduced fecal P content (Bregitzer *et al.* 2008).

Phytic acid is a cation chelator and in grains reduces bioavailability of essential minerals such as iron and zinc in human diet. A range of mutants with low phytic acid have been developed in different grain crops, including rice (Liu *et al.* 2007, Rutger *et al.* 2004, Zhao *et al.* 2008), barley (Raboy *et al.* 2015, Ye *et al.* 2011) and maize (Pilu *et al.* 2003), as well as in legume crops such as soybean (Sebastian *et al.* 2000) and common bean (Campion *et al.* 2009, Cominelli *et al.* 2018, Raboy 2016). A review of the performance, bioavailability of minerals and nutritional quality of several low phytic acid cereal mutants showed their exceptional value (Raboy 2016).

Improvements in oil quality in soybean (Fehr and Hammond 1998, Hammond and Fehr 1983), canola (Landge *et al.* 2009) and peanut (D'Souza *et al.* 2009, Kharkwal and Shu 2009, Mondal *et al.* 2011) have also been achieved through mutation breeding. Sequencing has revealed that a G to A transition was responsible in one such peanut mutant (Mondal *et al.* 2011). Work towards reduction of allergens from peanut and improving oleic to linoleic acid ratio using a TILLING approach was undertaken by Knoll *et al.* (2011).

Increased seed size in the TG series of peanut mutants in India has contributed immensely to the confectionery industry (D'Souza *et al.* 2009, Kharkwal and Shu 2009). The mutant barley cultivars 'Golden Promise' and 'Diamant' have added billions of dollars to the value of the brewing and malting industries in

Europe. For its high malting quality, 'Golden Promise' was in wide cultivation in the UK and Ireland in the 1960s and 1970s. Even 30 years after release, it is still popular in Scotland and is estimated to have contributed US\$417 million to Scotland's brewing industry alone (Ahloowalia *et al.* 2004).

High quality durum wheat mutants in Italy and bread wheat in India and Pakistan have added value to the grain industry of those countries (Ahloowalia *et al.* 2004, Kharkwal and Shu 2009).

Using high performance thin layer chromatography to screen low glycoalkaloid mutants in a population derived from gamma ray irradiated bud eye pieces of potato, Love *et al.* (1996) isolated three mutant lines with high potential for the potato chip industry. A high amylose sunflower mutant line has been isolated by seed irradiation followed by screening in the M2 population and subsequent clonal multiplication and evaluation (Ceballos *et al.* 2008).

Using a mix of mutation breeding and biotechnological approaches, wheat and barley cultivars suited for celiac patients are also being developed (von Wettstein 2009). This is achieved by inhibition of demethylation of gene promoters that control the production of gliadin-type proteins during endosperm development by mutation, without affecting the high molecular weight glutenin formation as the latter is sufficient to produce the molecular structure of elastic fibrils that form dityrosine cross-links during dough formation and baking (von Wettstein 2009). Celiac patients are sensitive to epitopes of gliadin-type prolamins which are of no importance to baking because high molecular weight glutenin alone is sufficient to produce high quality bread. A viable mutant of barley with such a mutation has already been obtained (von Wettstein 2009). For hexaploid wheat the current strategy is to use TILLING populations to discover mutations in the 5-methylcytosine DNA deglycosylase genes in the three homeologous genomes (Slade *et al.* 2005, Slade and Knauf 2005, von Wettstein 2009).

### **Herbicide tolerance**

Herbicide tolerance in crop cultivars is a feature sought after in modern agriculture, particularly when cultivating dwarf and semi-dwarf compact cultivars, which have poor competing ability with weeds. Mutations in different genes in plants can make them tolerant of different groups of herbicides that act in different ways. Some selected examples from many herbicide tolerant mutants induced using chemical and physical mutagens, and through seed treatment and treatment of *in vitro* embryogenic cultures in contrasting plant families are presented in Table 4.

### **CONCLUSIONS**

Mutations have been the cornerstone of plant evolution and diversification. Increasing number of plant species subjected to WGS has allowed deciphering the role of mutations, particularly that of duplications within the genome and WGD, making possible for plants to colonise the earth surface successfully. Since the first artificially induced mutations almost a century ago and over a half a century of practical application of the technology, induced mutations have played an important role in plant breeding, contributing to increased food production in both developed and developing economies. Both ionising radiation and chemical mutagenesis are continued to be used in practical mutation breeding and novel methods such as ion beam and space mutations have added to the arsenal of available sources for mutagenesis. Classical mutation breeding continues to be used for the benefit of communities in parallel with application of modern genomic tools for mutation induction and discovery. Mutation breeding has proven flexible, workable and ready to use on any crop if objectives and selection methods are clearly identified. A range of mutagens are at our disposal to induce mutations from the single nucleotide level to the genome level. Induced mutations have not only played an unprecedented role in developing new crops, new cultivars and novel products from existing crops, but also increasingly contribute to our understanding of gene function and biochemical pathways. Along with newly emerged "omics" techniques, induced mutations are

**Table 4:** Selected examples of herbicide tolerant mutants induced in dicotyledonous and monocotyledonous annual and perennial crops

Species	Mutation	Mutagen	Method	Reference
<i>Lupinus angustifolius</i> L.	Metribuzin tolerance (Triazinone group – photosystem II inhibitor)	1 mM sodium azide (pH 3), 3 h	Soak seeds 8 h, 20° C then treat	Si <i>et al.</i> (2009)
<i>Helianthus annuus</i> L. maintainer line BTK47	Imidazolinone resistance (acetolactate synthase inhibitor)	0.25% (v/v) EMS <sup>a</sup> (pH 7), 15 h, 25°C.	Post-treatment soak seeds for 30 min in 1.5% (w/v) sodium thiosulphate	Sala <i>et al.</i> (2008)
<i>Lens culinaris</i> F <sub>3</sub> bulk	Imidazolinone resistance	EMS	N/A	Slinkard <i>et al.</i> (2007)
<i>Helianthus annuus</i> L. HA89B maintainer inbred line	Sulfonylurea tolerance (acetolactate synthase inhibitor)	EMS 30-35 mM (pH 5.6) – 18 h	After treatment soak seeds in 1.5% (w/v) sodium thiosulphate, 1min	Gabard and Huby (2001)
<i>Triticum aestivum</i> L.	Glyphosate tolerance (Inhibition of 5-enolpyruvyl shikimate-3 phosphate synthase)	EMS 0.4%, 16 h, 22° C	After treatment soak seeds in 10% (w/v) sodium thiosulphate, 5 min	Aramrak (2015)
<i>Triticum aestivum</i> L. ‘Hatcher’ winter wheat (PI 638512)	Fluazifop (an aryloxyphenoxy propionate - inhibitor of acetyl co-enzyme A carboxylase)	60 mM EMS; 2.27 kg seed soaked for 2.5 h in 5 L solution	Dry in fume hood after treatment before sowing	Ostlie <i>et al.</i> (2015)
<i>Gossypium hirsutum</i> L. Four US upland cotton cultivars	Imidazolinone tolerance	EMS 2.45% (v/v) 2 h treatment	Seeds soaked in water for 16 h with aeration before treatment	Bechere <i>et al.</i> (2010)
<i>Saccharum officinarum</i> L. cv. ‘N12’	Imidazolinone tolerance	10 ml of 16mM EMS solution per 0.2 g embryogenic callus, 4 h.	3 days recovery followed by herbicide incorporation (increasing concentration over time) to media for selection.	Koch <i>et al.</i> (2012)
<i>Lycopersicon esculentum</i> L. cv. ‘M82’	Imidazolinone resistance	10 h soak in water, EMS 3.5% under 80 rpm shaking, 12 h - (50% germination reduction dose)	30 m wash under running tap water, dry and plant	Dor <i>et al.</i> (2016)
<i>Zoysia japonica</i> Steud.	Glyphosate resistance	Gamma-rays (50-70 Gy)	<i>In vitro</i> cultures irradiated	Lee <i>et al.</i> (2008)
<i>Camelina sativa</i> L.	Imazethapyr, sulfosulfuron, and flucarbazone tolerance (All acetolactate synthase inhibitors)	Soak seeds overnight in EMS 0.3% in 0.1 M phosphate buffer, pH 7.5.	IM1 and IM6 mutants derived from cv. ‘Cheyenne’, IM10 and IM 18 from cv. ‘Calena’	Walsh <i>et al.</i> (2012)

<sup>a</sup>EMS – Ethyl methanesulfonate

contributing to the development of newly emerging subject of systems biology.

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