Chapter 1
Mutagenesis for Crop Breeding and Functional Genomics

Joanna Jankowicz-Cieslak, Chikelu Mba, and Bradley J. Till

Abstract Genetic variation is a source of phenotypic diversity and is a major driver of evolutionary diversification. Heritable variation was observed and used thousands of years ago in the domestication of plants and animals. The mechanisms that govern the inheritance of traits were later described by Mendel. In the early decades of the twentieth century, scientists showed that the relatively slow rate of natural mutation could be increased by several orders of magnitude by treating *Drosophila* and cereals with X-rays. What is striking about these achievements is that they came in advance of experimental evidence that DNA is the heritable material. This highlights one major advantage of induced mutations for crop breeding: prior knowledge of genes or gene function is not required to successfully create plants with improved traits and to release new varieties. Indeed, mutation induction has been an important tool for crop breeding since the release of the first mutant variety of tobacco in the 1930s. In addition to plant mutation breeding, induced mutations have been used extensively for functional genomics in model organisms and crops. Novel reverse-genetic strategies, such as Targeting Induced Local Lesions IN Genomes (TILLING), are being used for the production of stable genetic stocks of mutant plant populations such as *Arabidopsis*, barley, soybean, tomato and wheat. These can be kept for many years and screened repeatedly for different traits. Robust and efficient methods are required for the seamless integration of induced mutations in breeding and functional genomics studies. This chapter provides an overview of the principles and methodologies that underpin the set of protocols and guidelines for the use of induced mutations to improve crops.

Keywords Mutation breeding • Reverse-genetics • Forward-genetics • Phenotyping • Genotyping • Technology packages

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1.1 Inducing Genetic Variation

The genetic improvement of crops is a crucial component of the efforts to address pressures on global food security and nutrition (Ronald 2011). It is estimated that food production should be at least doubled by the year 2050 in order to meet the needs of a continually growing population (Ray et al. 2013; Tester and Langridge 2010; FAO 2009). The availability of heritable variation is a prerequisite for genetic improvement of crops. Where sufficient variation does not exist naturally, it can be created through either random or targeted processes (Fig. 1.1). Aside from recombination, the treatment of plant materials with chemical or physical mutagens is the most commonly reported approach for generating novel variation. While various mutagens have different effects on plant genomes, and some positional biases have been reported, irradiation and chemical mutagenesis are generally considered random mutagenesis as the location of DNA lesions cannot be effectively predicted in advance (Greene et al. 2003). The effect of different mutagens on the DNA sequence also varies with mutagen type and dosage. Once sufficient genetic variation is induced, the next step is to select materials that have the desired altered traits (see Fig. 1.1 and Sects. 1.2 and 1.3).

1.1.1 Practical Considerations in Induced Crop Mutagenesis

Mutation breeding is a three-step process consisting of (a) inducing mutations, (b) screening for putative mutant candidates and (c) mutant testing and official release (Fig. 1.2). The last step tends to be standardised in specific countries and is not an area where research and development can (easily) improve efficiencies. While not trivial, mutation induction has been widely used and highly successful in most species. Screening of mutants and selection of desired variants remain the most intensive step. Incredible advances have been made in the field of phenomics over the past 5 years, however, phenotyping remains more specialised and labour intensive than genotypic selection (Fiorani and Schurr 2013; Cobb et al. 2013).

The choice of which type of mutagen to use for mutation breeding is often based on past successes reported for the species and other considerations such as the availability of mutagens, costs and infrastructure (Bado et al. 2015; Mba 2013; MVD 2016). Mutant varieties produced with ionising radiation, specifically gamma rays, predominate in the database of registered mutant varieties (MVD 2016). This may be due primarily to the active promotion of the use of gamma irradiation by the Food and Agriculture Organisation of the United Nations and the International Atomic Energy Agency (FAO/IAEA) Joint Programme, but also may be biologically significant as physical mutagens tend to induce larger genomic aberrations than some chemical mutagens, and more dominant or more easily observable traits could be created at a higher frequency (Jankowicz-Cieslak and Till 2015). Standardised protocols and general considerations for induced mutations in seed
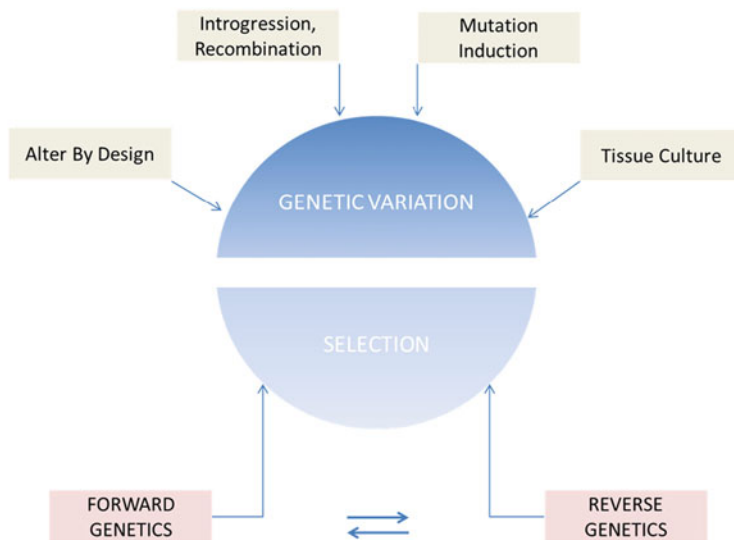
Fig. 1.1 Crop improvement strategies based on the generation and harnessing of genetic variation. There are many methods to introduce novel genetic variation into a specific line. The most common is through outcrossing, whereby introgression and recombination generate new combinations of alleles. This may include wide intraspecific and interspecific crosses. Passaging of cells through tissue culture has also been used to generate what is known as somaclonal variation. 'Alter by design' refers to any method whereby genetic variation is induced through thoughtful modifications. These include methods such as transgenics or genome editing (see Chap. 7). Mutagenesis provides a low-cost means to rapidly generate novel variation. The next step is to select plants that have the desired mutation or phenotype. Here, the researcher can choose between forward and reverse-genetic approaches depending on prior knowledge of genes and hypotheses of gene function. In addition to direct traditional phenotyping, the emerging fields of genomics and phenomics offer opportunities for more precise breeding and large gains in efficiencies while reducing the time for recovery of desired variants (see Sects. 1.2 and 1.3). Figure adapted from Novak and Brunner (Novak and Brunner 1992)

and vegetatively propagated plants using the physical mutagen (gamma rays) and the chemical mutagen (ethyl methanesulfonate, EMS) have been previously discussed (Lee et al. 2014; Bado et al. 2015; Till et al. 2006; Mba et al. 2010). Chapters 2, 3, 4 and 6 of this book describe chemical and/or physical mutagenesis protocols for obligate vegetatively propagated banana (*Musa acuminata*), facultative vegetatively propagated Jatropha (*Jatropha curcas*) and seed-propagated barley (*Hordeum vulgare*).

A major bottleneck in plant mutation breeding is the imperative of generating and evaluating large mutant populations in order to increase the chance of identifying a desirable variant. Efforts are devoted to the dissociation of chimeras, also known as mosaics or sectoral differences, whereby cells of different genotypes exist side by side in the tissues of the same mutant plant. This is straightforward in sexually produced crops owing to the fact that single cells in the form of gametes are the basis for the next generation, thus resolving any chimeras. For vegetatively
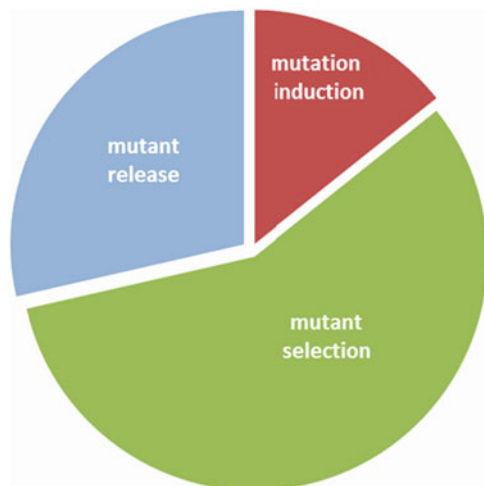
Fig. 1.2 A three-step mutation breeding scheme for direct release of improved crops. Each part is drawn proportional to the estimated time needed for development of a seed-propagated cereal (7–10 years). The first step is mutation induction which may take up to a year. The most time consuming and complicated step is mutant selection. Several years are typically needed to identify useful traits that are stable through propagation cycles. The third step, mutant varietal release, follows standardised procedures of the country where the material is grown. This often requires multilocational trials with farmer involvement. While the timing of this may vary, it is usually a shorter duration than the selection and testing phase. The procedure becomes longer and more complicated if the selected mutants are used as pre-breeding material in hybridisations (see Chap. 11)

propagated crops, several cycles of regeneration may be required to produce solid homohistonts or genotypically homogeneous material (van Harten 1998; Mba et al. 2009). One way to avoid chimerism in vegetatively propagated species is to mutagenise individual cells that can regenerate into plants, either using cell suspensions or (embryogenic) callus (van Harten 1998). Protocols for these strategies are provided in Chaps. 4 and 5. These approaches have been less often used than those involving multicellular organs and tissues, and so there is less information available on the possibility of chimerism at the DNA sequence. It is interesting to speculate on the fate of induced DNA modifications in single cells. For example, EMS mutagenesis results in alkylation, whereby the original base is not physically altered, but the mutation is only fixed due to an error in replication of the affected base. Here, two daughter cells could be produced with distinct genotypes.

1.1.2 Developing Crop Varieties Using Induced Mutations

Once a mutant population has been developed, the next steps of the mutation breeding process mirror traditional breeding procedures (Fig. 1.3). One issue to

consider is in which generation the selection for desirable putative mutants could begin. Depending on the density of mutations, selection of stable phenotypes in the M_2 may be difficult. This is due to the potential confounding factors of combinations of deleterious lesions (which affect the function of different proteins) and epistasis. One consequence of selecting phenotypes too early is that the observed trait may be lost in segregation in subsequent generations as non-linked alleles assort independently. The researcher may choose to accept this risk and select everything of interest in the first non-chimeric generation (M_2 for seed) for further
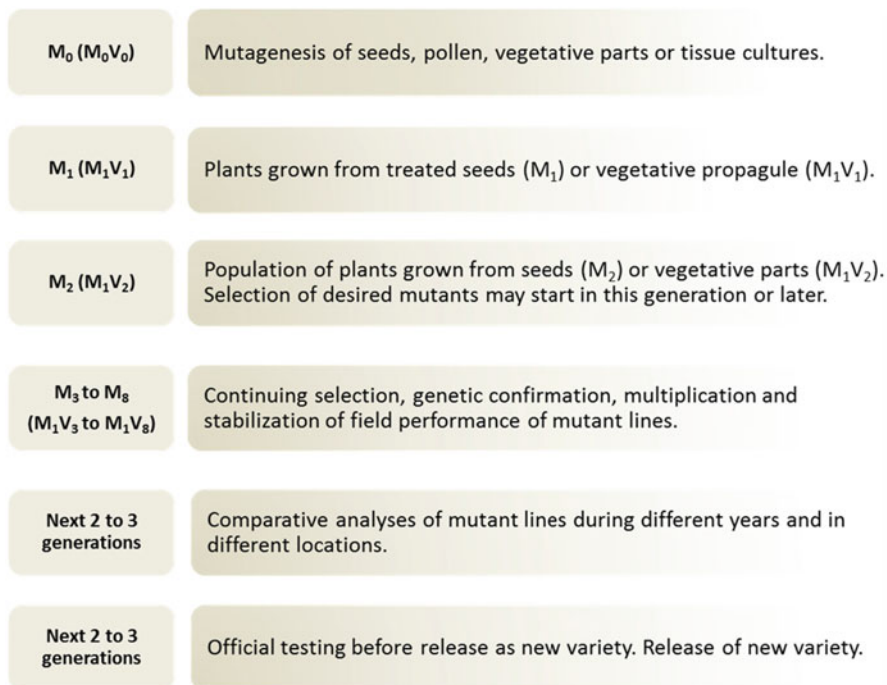
M_0 (M_0V_0)	Mutagenesis of seeds, pollen, vegetative parts or tissue cultures.
M_1 (M_1V_1)	Plants grown from treated seeds (M_1) or vegetative propagule (M_1V_1).
M_2 (M_1V_2)	Population of plants grown from seeds (M_2) or vegetative parts (M_1V_2). Selection of desired mutants may start in this generation or later.
M_3 to M_8 (M_1V_3 to M_1V_8)	Continuing selection, genetic confirmation, multiplication and stabilization of field performance of mutant lines.
Next 2 to 3 generations	Comparative analyses of mutant lines during different years and in different locations.
Next 2 to 3 generations	Official testing before release as new variety. Release of new variety.

Fig. 1.3 Traditional mutation breeding scheme. Each row describes the steps for a specific generation. The generation nomenclature starts with M_0 for seed or pollen mutagenesis and M_0V_0 for vegetative organs, where M stands for the meiotic and V for the vegetative generation. All materials are labelled with a '0' prior to mutagenesis and with a '1' after mutagenesis is performed. The first generation is not suitable for evaluation when multicellular material is mutagenised because resulting plants will be genotypically heterogeneous (chimeric). The first non-chimeric (homohistont) generation in a seed-mutagenised and seed-propagated material is the M_2. It may take several cycles to make a vegetatively propagated material genotypically homogeneous and to stabilise the inheritance of mutant alleles. Screening and selections can begin as early as the first non-chimeric generation. Subsequent generations typically involve selection and evaluation of mutant phenotypes to ensure that the traits are reproducible. Once this is complete, the materials can enter trials for varietal release. Alternatively, materials can be used as parents in breeding programmes. Officially released mutant crop varieties which are reported to the Joint FAO/IAEA Programme are recorded in the searchable Mutant Variety Database (MVD 2016). According to the MVD, approximately 62 % of all mutant varieties are directly released. Figure adapted from Novak and Brunner (Novak and Brunner 1992)

characterisation (see Chap. 9). On the other hand, when considering reverse-genetic strategies, it is often preferable to employ molecular screens on the first non-chimeric generation in order to maximise the discovery of unique mutations (Jankowicz-Cieslak and Till 2015). Also of critical importance is the method employed to select desirable phenotypes. While phenomic strategies have been rapidly developing in recent years (Cobb et al. 2013), the diversity of physiological parameters, disease responses and morphological variations from crop to crop complicates the task of developing standardised species-independent protocols, as can be done with most genomic screening tools (see Sect. 1.2).

1.1.3 Elite Crop Varieties Developed Through Induced Mutations

Shortly after scientists discovered that mutations could be induced through work on *Drosophila* (Muller 1927) and cereals (Stadler 1929, 1928a, b), plant breeders started using this as a tool to develop novel varieties. The first example was a mutant of tobacco called 'Chlorina' that was developed through the X-ray irradiation of floral buds in the 1930s (Tollenaar 1934, 1938; Konzak 1957; Coolhaas 1952). The Mutant Varieties Database maintained by the Joint Programme of the Food and Agriculture Organisation of the United Nations and the International Atomic Energy Agency (Joint FAO/IAEA) in Vienna, Austria, has searchable data for over 3220 crop varieties that have been developed using induced mutations and are being grown in different countries of the world (Fig. 1.4 and (MVD 2016)). Nearly 80 % of these crop varieties are seed propagated, almost half of which (48 %) are cereals.

Ahloowalia et al. (2004) and Kharkwal and Shu (2009) provided overviews of the contributions of these mutant crop varieties to food security and nutrition and economic wellbeing. Widely cultivated rice varieties in Australia, China, India, Pakistan and Thailand; sunflower and peppermint in the USA; barley in many countries of Europe; sorghum in Mali; and several ornamental plants in India, the Netherlands and Germany are a few such examples. The high-yielding and dwarf mutant cultivars of barley, 'Diamant' and 'Golden Promise', for instance, and their progenies are credited with additional billions of dollars in revenues for the brewing and malting industries in Europe (Ahloowalia et al. 2004). Other examples include most of the varieties of durum wheat grown in Italy for pasta and marketed worldwide; the Rio Star grapefruit in the USA; the Japanese pear variety, 'Gold Nijesseiki'; and the cotton variety, NIAB78, in Pakistan.

It is difficult to estimate the precise extent to which novel alleles created through mutagenesis have been used in developing superior crop varieties worldwide. From a practical standpoint in most parts of the world, they are treated like any allele that the breeder may knowingly or unknowingly incorporate into a new improved cultivar.
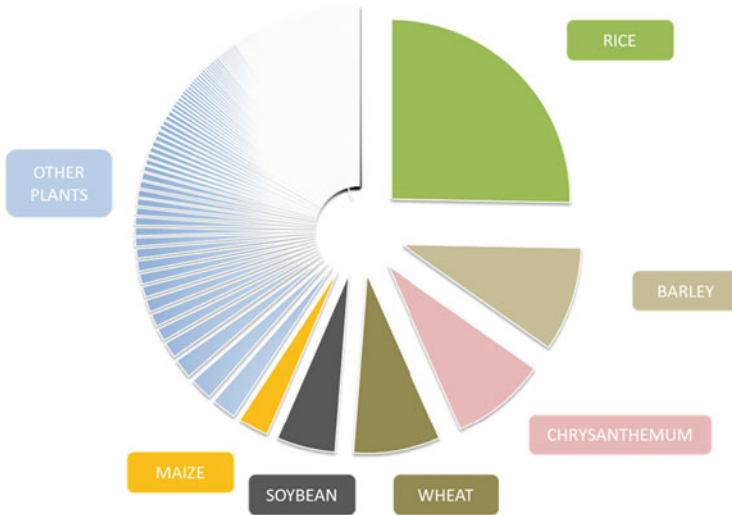
Fig. 1.4 Pie chart representing officially registered mutant crop varieties. The Mutant Variety Database contains 3222 entries out of which 2456 are seed propagated and 367 vegetatively propagated plants. The above grouping is based on the common name of the entry. The top six are rice, barley, chrysanthemum, wheat, soybean and maize. Data comes from (MVD 2016) accessed on May 4th, 2016

1.2 Phenotypic Screening

The origin of agriculture can be traced back to more than 10,000 years ago when the first crops were domesticated in the Fertile Crescent (Brown et al. 2009). The identification (phenotyping) and deliberate selection of off-type plants (i.e. spontaneous mutants) by the then hunters and gatherers were the initial efforts at plant breeding. The first plants to undergo domestication are thought to be the cereals wheat, barley, millet and emmer (Sang 2009). Early phenotypers selected plants with increased grain size and loss of seed shattering. They created and improved crops without any knowledge of why the selected differences occurred or were heritable. The first written reports of the earliest description of the selection of (presumably) spontaneous mutants appear to date back to around 300 BC when selection of plants with abnormal but improved traits were described in an ancient Chinese book, 'Lulan' (Shu et al. 2012). Among the improved characters were 'days to maturity' and other easily visible traits in cereal crops. The term 'mutation' was coined much later by Hugo de Vries to describe sudden genetic change in higher plants which was stably inherited through many years (de Vries 1901). Spontaneous mutants remain valuable sources of diversity and variation, but many years of intensive breeding has resulted in the narrowing of the genetic base of many crop species necessitating the need to create new variation through means such as induced mutations.

Accurate plant phenotyping remains as critically important today as it has ever been and is arguably the major bottleneck in plant breeding (Fiorani and Schurr 2013). This is especially true in plant mutation breeding where a novel trait may appear only once in a population of several thousand mutant lines. Therefore, high-throughput and cost-efficient screening methods are required for the rapid identification and characterisation of putative mutants.

1.2.1 Phenotypic Traits Developed Through Plant Mutation Breeding

Plant phenotyping can broadly be described as the evaluation of plant traits defined by the researcher (breeder) and may include yield, quality and resistance to biotic/abiotic stresses. The list can be further extended depending on the need and question asked. The Mutant Variety Database contains released and registered mutant plants with improved traits (characters) in five main categories: 'agronomic and botanic traits', 'quality and nutrition traits', 'yield and contributors', 'resistance to biotic stresses' and 'tolerance to abiotic stresses' (Fig. 1.5 and Table 1.1). For the 3222 officially registered mutants, 5569 improved characters are listed, implying that many mutants show several improved traits. For instance, higher yield is observed when a plant has improved resistance to abiotic or biotic stresses. Such a mutant, therefore, will have more than one improved character. It remains difficult to even speculate on the number of mutated alleles and genes that are causing the induced variation. Genomic technologies will allow the elucidation of mutant alleles causing altered traits (see Sect. 1.3).

Interestingly, the majority (48 %) of released mutant varieties that are registered in the Mutant Variety Database are characterised by improved agronomic and botanic traits. This could be due to the fact that botanic and agronomic traits are easily observable, and for most of them, no specialised equipment is needed for screening. The least represented mutants are in the biotic and abiotic stresses category. It is notable that these characters, though complex and difficult to screen for, are important breeding objectives. Examples of officially released mutant varieties in the five trait categories are listed in Table 1.1.

There remains a clear need to develop methods and protocols to enhance the efficiency of the mutation breeding process. The protocols in this book aim to do this, but it remains an incredibly challenging task. Every step of the procedure can differ depending on the parental genotype, propagation mode (seed versus vegetatively), the trait of interest which needs to be improved and available facilities. For example, Near-Infrared Reflectance Spectroscopy (NIRS) is a method that can be used to screen for seed composition. Traditional methods used a destructive approach which is suitable for characterising an advanced mutant line where many seeds are available. Screening is rapid, and non-destructive methods that measure whole seed allow NIRS to be used as a fast prescreen of large mutant
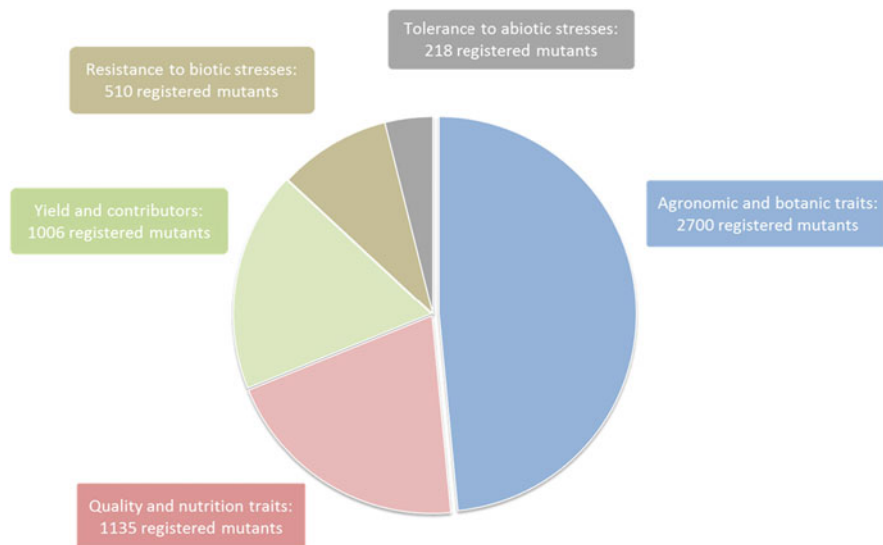
Fig. 1.5 Mutants registered in the MVD classified according to improved characters (traits). In total, improved characters are described 5569 times for 3222 varieties. These are classified in five general categories: 'agronomic and botanic traits' (48 %), 'quality and nutrition traits' (20 %), 'yield and contributors' (18 %), 'resistance to biotic stresses' (9 %) and 'tolerance to abiotic stresses' (4 %). Agronomic and botanic traits include maturity, flowering time and plant structure. Data comes from (MVD 2016) accessed on May 4, 2016

populations. Protocols for both destructive and non-destructive screening of rice are provided in Chap. 12. Calibration standards can be applied to NIRS spectra to evaluate seed components such as protein content. Once interesting mutants are identified in a rapid prescreen, detailed characterisation can be undertaken. Proteomic analysis allows a detailed cataloguing of the effect of genetic variation on the collection of expressed proteins in grains or tissues. Chapter 13 of this book provides detailed methods for protein analysis in seed and leaves of *Jatropha curcas*. Digital imaging is another non-destructive method that can be adapted for phenotypic evaluation of morphological variations induced by treatment with mutagens. Root architecture, for example, is an important component in abiotic stress responses such as drought. A low-cost approach for digital analysis of root traits is provided in Chap. 10.

Table 1.1 Examples of released improved varieties registered in the Mutant Variety Database under five main trait categories

Trait category	Mutant's name (*species*)	Description[a]	Development type [b]	References
Agronomic and botanic traits	'Above' (*Triticum aestivum* L.)	Awned, white glumed, early maturing and semidwarf	Treatment of seed with chemical mutagen, sodium azide (NaN3)	Newhouse et al. (1992)
Quality and nutrition traits	'Aldamla' (*Prunus avium* L.)	Compact growth habit (70–80 %), long petioles and improved fruit quality	Irradiation of dormant buds with gamma rays	Kunter et al. (2012)
Resistance to biotic stresses	'Akita Berry' (*Fragaria x ananassa*)	Improved resistance to black leaf spot disease (*Alternaria alternata*)	Somaclonal mutation by meristem culture	MVD (2016)
Tolerance to abiotic stresses	'Maybel' (*Lycopersicon esculentum* M.)	Very high performance under drought conditions	Treatment of seed with gamma rays	MVD (2016)
Yield and contributors	'Early Blenheim' (*Prunus armeniaca* L.)	Early maturity, higher yield, large fruits and self-compatible pollen	Treatment of dormant scions with thermal neutrons (thN)	Sigurbjoernsson and Micke (1974)

[a]Some mutants listed have more than one character type
[b]No molecular characterisation reported concerning novel variation causing phenotype

1.3   Genotypic Screening of Mutant Plants

1.3.1   Genotypic Methods

Plant genotyping can be broadly considered as any experimental assay that aims to evaluate differences in the nucleotide sequence within or between species. This is an especially powerful approach because nucleotide variation is the major contributor to heritable phenotypic variation. Methods to uncover nucleotide variation also provide important information on plant evolution and enable efficient selections that avoid the confounding effects of genotype by environment (GxE) interactions (Annicchiarico 2002). Protocols for genomic DNA acquisition and evaluation have been improving to the point where resequencing of hundreds to thousands of plant genomes is now a reality (Weigel and Mott 2009).

1.3.1.1   Lower-Cost Mutation Discovery and Genotyping Methods

The risk of new technologies, however, is that they tend to be expensive and require a high level of technical expertise. New tools, therefore, are not available to all

laboratories. Yet many powerful methods can be developed that are lower cost and suitable for laboratories with varying infrastructure. One example is the starting point of all genotyping experiments: the extraction of DNA. While long-term storage of plant tissues prior to DNA extraction often involves the use of liquid nitrogen and −80 °C freezers, these can be avoided by desiccating and storing leaf material in silica gel at room temperature (Till et al. 2015). Extraction of high-quality genomic DNA from leaf material is typically performed using expensive kits or with more manual methods that require toxic organic chemicals such as the CTAB method. These can be avoided by using the protocol described in Chap. 14 of this book. DNA is extracted by binding to silica in the presence of chaotropic salts. This mirrors the chemistry used in expensive kits but at about only 10 % of the price. Importantly, therefore, high-quality genomic DNA can be extracted without specialised equipment for tissue grinding and without the use of any toxic organic compounds that require specialised waste disposal.

Low-cost methods do not end with the extraction of genomic DNA. The process of altering the expression or activity of a gene in order to evaluate its function in vivo is known as reverse-genetics. This term was coined because it is essentially the reverse of the process of forward-genetics which starts with a phenotype and ends with a gene sequence. While endogenous transposons have been used for gene disruptions in some crops such as maize and rice, a major development came in the late 1990s with a reverse genetic approach known as TILLING that uses induced mutations (Meeley and Briggs 1995; McCallum et al. 2000; Hirochika 2001; Conrad et al. 2008; Hunter et al. 2014). TILLING, short for Targeting Induced Local Lesions IN Genomes, typically utilises mutagens that induce a high density of induced mutations randomly throughout the genome (Kurowska et al. 2011; Greene et al. 2003; Jankowicz-Cieslak et al. 2011). A population of between 3000 and 6000 mutant lines can be developed that contains multiple mutations in every gene in the genome. A library of DNA and seed can be prepared and used as a resource for many years. In traditional TILLING, the DNA library is screened by PCR and enzymatic mismatch cleavage to identify mutations in target genes of choice. The entire TILLING process can be made low cost. In addition to low-cost methods for DNA extraction found in Chap. 14, Chap. 15 describes a rapid method for the extraction of single-strand-specific nucleases for TILLING and other applications that costs less than 1 cent per assay. Standard agarose gels can be used as a readout platform for mutation discovery.

1.3.1.2 Higher-Throughput Genotyping and Mutation Discovery Methods

Where budgets permit, next-generation sequencing technologies offer significant gains in screening throughput over low-cost methods. For example, the TILLING by sequencing protocol described in Chap. 20 provides a three-dimensional pooling strategy for 768 individuals and simultaneous mutation discovery in many gene targets (Tsai et al. 2011). The use of advanced tools is not limited to reverse-genetics. The majority of officially released mutant crop varieties are produced

from forward-genetic screens from plant materials treated with ionising radiation (MVD 2016). Genomic techniques promise to greatly enhance the efficiency of traditional forward mutation breeding that has been a mainstay for over 70 years. The challenge remains to determine if a population truly harbours a high density of desirable mutations. While visual evaluation of M_1 plants as described above is advantageous in that it is rapid and low-cost, it is known that variations observed in the M_1 do not represent heritable DNA mutations (Preuss and Britt 2003). Therefore, mutation density need not correlate with phenotypic variations observable in the M_1. Next-generation sequencing technologies now provide rapid methods for the evaluation of mutation density and spectra in the M_2 generation. Many plant genomes are prohibitively large to consider whole-genome sequencing of the requisite number of plants for all but the very highly funded laboratories. Reduced representation genome sequencing offers a solution. Chapter 19 provides a protocol for Restriction Enzyme Sequence Comparative Analysis (RESCAN) where a fraction of a plant genome can be sequenced for discovery of induced point mutations. Here, tens of millions of base pairs can be sequenced from each mutant plant to recover sufficient mutations for a suitable estimation of mutation density.

1.3.1.3 Cloning Mutant Alleles Causative for Improved Traits

The same protocols described above can be adapted for another major challenge of forward mutation breeding: the identification and cloning of mutations causing the improved trait. With smaller genome plants, it is possible to sequence whole genomes and clone genes by associating co-segregation of genotype to phenotype (Schneeberger et al. 2009; Cuperus et al. 2010). An approach known as MutMap has been described for cloning EMS-induced alleles in rice using a bulked segregant strategy, and the method further adapted so that alleles can be cloned without outcrossing (Abe et al. 2012; Fekih et al. 2013). This is much more challenging in larger genome crops due to throughput and cost limitations of whole-genome sequencing. Targeted capture-resequencing methods offer a way for reduced representation genome sequencing of specific regions designed by the researcher. Coding sequences are an excellent choice when mutations affecting gene function are sought. Henry and colleagues describe exome capture methods to recover EMS-induced mutations in rice and wheat (Henry et al. 2014). With large genomes like wheat, this approach allows massive enrichment of functional regions of the genome and makes applications such as MutMap feasible, so long as causative mutations lie within regions that the researcher has selected for sequencing. To date, the majority of efforts have focused on recovery of point mutations such as those induced by treatment with the chemical mutagen EMS. Less is known about the effects of mutagens such as gamma irradiation, but recent experiments suggest that mutagen causes primarily large genomic deletions. For example, deletions of 1.2 million base pairs and 232,000 base pairs were recovered in *Zea mays* treated with gamma irradiation (Yuan et al. 2014). In sorghum, deletions ranging between 100,000 kb and 700,000 kb were recovered in materials treated with 75 and 300 Gy

(B.J Till, I.M. Henry and L. Comai, unpublished). In contrast to what is emerging from gamma irradiation, whole-genome sequencing studies of fast neutron-irradiated rice suggest a broader spectrum of mutations (Li et al. 2016).

The presence of large genomic deletions may make the task of cloning much easier. For example, a diploid such as sorghum with a ~ 730 Mbp genome that is treated with EMS may harbour 3000 induced mutations making the job of finding the one mutation causing the trait difficult. The same genome treated with gamma irradiation may only be able to accumulate a small number of large genomic indels. Thus, identifying the mutation causing the phenotype is severalfold easier. Discovery of large genomic indels via sequencing may also prove more efficient as lower depth of coverage is needed for accurate variant calling compared to SNPs, and therefore more samples can be screened per run. This approach has been used to catalogue gamma-induced mutations created through irradiation of pollen from poplar (Henry et al. 2015). Continued improvement in sequencing technologies suggests that cloning both SNP and large indel mutations will become more common in the near future. This will make valuable mutant alleles available to breeders for marker-assisted introgression into elite germplasm.

1.4 Conclusion

There is little controversy that growing pressures on agricultural productivity such as increasing population, reduction of arable land and new and geographically shifting biotic and abiotic stresses demand serious attention and innovative approaches. Genetic improvement of crops is fundamental to long-term success, and a combination of novel developments and translational science is required. We predict that induced mutagenesis will remain an important tool for the breeder as it is a rapid and relatively low-cost approach to generate novel alleles and phenotypes. Further, new technologies will enable determination of mutant alleles used to create successful mutant varieties and will shed light on gene function and crop productivity.

References

Abe A, Kosugi S, Yoshida K, Natsume S, Takagi H, Kanzaki H, Matsumura H, Mitsuoka C, Tamiru M, Innan H, Cano L, Kamoun S, Terauchi R (2012) Genome sequencing reveals agronomically important loci in rice using MutMap. Nat Biotechnol 30(2):174–178

Ahloowalia BS, Maluszynski M, Nichterlein K (2004) Global impact of mutation-derived varieties. Euphytica 135(2):187–204

Annicchiarico P (2002) Genotype X Environment interaction: challenges and opportunities for plant breeding and cultivar recommendations. Food Agriculture Organization of the United Nations, FAO, Rome, Italy

Bado S, Forster BP, Till BJ, Nielen S, Ghanim AMA, Lagoda PJL (2015) Plant mutation breeding: current progress and future assessment. Plant Breeding Reviews

Brown TA, Jones MK, Powell W, Allaby RG (2009) The complex origins of domesticated crops in the Fertile Crescent. Trends Ecol Evol 24(2):103–109

Cobb JN, Declerck G, Greenberg A, Clark R, McCouch S (2013) Next-generation phenotyping: requirements and strategies for enhancing our understanding of genotype-phenotype relationships and its relevance to crop improvement. Theor Appl Genet (Theoretische und angewandte Genetik) 126(4):867–887

Conrad LJ, Kikuchi K, Brutnell TP (2008) Transposon tagging in cereal crops. In: The handbook of plant functional genomics. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, pp. 267–290

Coolhaas C (1952) Large-scale use of F1 hybrids in "Vorstenlanden" tobacco. Euphytica 1(1):3–9

Cuperus JT, Montgomery TA, Fahlgren N, Burke RT, Townsend T, Sullivan CM, Carrington JC (2010) Identification of MIR390a precursor processing-defective mutants in Arabidopsis by direct genome sequencing. Proc Natl Acad Sci USA 107(1):466–471

de Vries H (1901) Die mutationstheorie. (English translation, 1910. The Open Court Publication, Chicago edn). Veit & Co., Leipzig

FAO (2009) FAO's Director-General on how to feed the World in 2050. Popul Dev Rev 35(4):837–839

Fekih R, Takagi H, Tamiru M, Abe A, Natsume S, Yaegashi H, Sharma S, Sharma S, Kanzaki H, Matsumura H, Saitoh H, Mitsuoka C, Utsushi H, Uemura A, Kanzaki E, Kosugi S, Yoshida K, Cano L, Kamoun S, Terauchi R (2013) MutMap+: genetic mapping and mutant identification without crossing in rice. PLoS One 8(7):e68529

Fiorani F, Schurr U (2013) Future scenarios for plant phenotyping. Annu Rev. Plant Biol 64:267–291

Greene EA, Codomo CA, Taylor NE, Henikoff JG, Till BJ, Reynolds SH, Enns LC, Burtner C, Johnson JE, Odden AR, Comai L, Henikoff S (2003) Spectrum of chemically induced mutations from a large-scale reverse-genetic screen in Arabidopsis. Genetics 164(2):731–740

Henry IM, Nagalakshmi U, Lieberman MC, Ngo KJ, Krasileva KV, Vasquez-Gross H, Akhunova A, Akhunov E, Dubcovsky J, Tai TH, Comai L (2014) Efficient genome-wide detection and cataloging of EMS-induced mutations using exome capture and next-generation sequencing. Plant Cell 26:1382–1397

Henry IM, Zinkgraf MS, Groover AT, Comai L (2015) A system for dosage-based functional genomics in poplar. Plant Cell 27(9):2370–2383

Hirochika H (2001) Contribution of the Tos17 retrotransposon to rice functional genomics. Curr Opin Plant Biol 4(2):118–122

Hunter CT, Suzuki M, Saunders J, Wu S, Tasi A, McCarty DR, Koch KE (2014) Phenotype to genotype using forward-genetic Mu-seq for identification and functional classification of maize mutants. Front Plant Sci 4:545

Jankowicz-Cieslak J, Huynh OA, Bado S, Matijevic M, Till BJ (2011) Reverse-genetics by TILLING expands through the plant kingdom. Emir J Food Agric 23(4):290–300

Jankowicz-Cieslak J, Till B (2015) Forward and Reverse Genetics in Crop Breeding. In: JM A-K, SM J, DV J (eds) Advances in plant breeding strategies: breeding, biotechnology and molecular tools, vol 1, 1 edn. Springer, Switzerland, pp. 215–240

Kharkwal MC, Shu QY (2009) The role of induced mutations in World Food Security. Induced plant mutations in the genomics era. Food and Agriculture Organization of the United Nations, Rome, Italy

Konzak CF (1957) Genetic effects of radiation on higher plants. Q Rev Biol 32(1):27–45

Kunter B, Bas M, Kantoglu Y, Burak M (2012) Mutation breeding of sweet cherry (Prunus avium L.) var. 0900 Ziraat. In: Shu Q, BP F, Nakagawa H (eds) Plant Mutation breeding and biotechnology. CAB International, Oxfordshire, pp. 453–463

Kurowska M, Daszkowska-Golec A, Gruszka D, Marzec M, Szurman M, Szarejko I, Maluszynski M (2011) TILLING—a shortcut in functional genomics. J Appl Genet 52(4):371–390

Lee LS, Till B, Hill H, Huynh O, Jankowicz-Cieslak J (2014) Mutation and mutation screening. In: RJ H, Furtado A (eds) Cereal genomics, Methods in molecular biology, vol 1099. Humana Press, Totowa, NJ, pp. 77–95

Li G, Chern M, Jain R, Martin JA, Schackwitz WS, Jiang L, Vega-Sanchez ME, Lipzen AM, Barry KW, Schmutz J, Ronald PC (2016) Genome-wide sequencing of 41 rice (Oryza sativa L.) mutated lines reveals diverse mutations induced by fast-neutron irradiation. Mol Plant 9:1078–1081

Mba C (2013) Induced mutations unleash the potentials of plant genetic resources for food and agriculture. Agronomy 3:200–231

Mba C, Afza R, Bado S, Jain SH (2010) Induced mutagenesis in plants using physical and chemical agents. In: Davey MR, Anthony P (eds) Plant cell culture: essential methods. Wiley, Chichester, UK, pp. 111–130

Mba CM, Afza R, Jankowcz-Cieslak J, Bado S, Matijevic M, Huynh O, Till BJ (2009) Enhancing genetic diversity through induced mutagenesis in vegetatively propagated plants. In: Shu QY (ed) Induced plant mutations in the genomics era. Food and Agriculture Organization of the United Nations, Rome, Italy, pp. 293–296

McCallum CM, Comai L, Greene EA, Henikoff S (2000) Targeting induced local lesions IN genomes (TILLING) for plant functional genomics. Plant Physiol 123(2):439–442

Meeley RB, Briggs SP (1995) Reverse genetics for maize. Maize Genet Coop Newsl 69:67–82

Muller HJ (1927) Artificial transmutation of the gene. Science 66(1699):84–87

MVD (2016) Mutant variety database. http://mvd.iaea.org/.

Newhouse KE, Smith WA, Starrett MA, Schaefer TJ, Singh BK (1992) Tolerance to imidazolinone herbicides in wheat. Plant Physiol 100:882–886

Novak FJ, Brunner H (1992) Plant breeding: induced mutation technology for crop improvement. IAEA Bull 4:25

Preuss SB, Britt AB (2003) A DNA-damage-induced cell cycle checkpoint in Arabidopsis. Genetics 164(1):323–334

Ray DK, Mueller ND, West PC, Foley JA (2013) Yield trends are insufficient to double global crop production by 2050. PLoS One 8(6):e66428

Ronald P (2011) Plant genetics, sustainable agriculture and global food security. Genetics 188 (1):11–20

Sang T (2009) Genes and mutations underlying domestication transitions in grasses. Plant Physiol 149(1):63–70

Schneeberger K, Ossowski S, Lanz C, Juul T, Petersen AH, Nielsen KL, Jorgensen JE, Weigel D, Andersen SU (2009) SHOREmap: simultaneous mapping and mutation identification by deep sequencing. Nat Methods 6(8):550–551

Shu QY, Forster BP, Nakagawa H (2012) Plant mutation breeding and biotechnology. CABI International, Cambridge, MA

Sigurbjoernsson B, Micke A (1974) Philosophy and accomplishments of mutation breeding. In: Polyploidy and induced mutations in plant breeding (Proceedings of two Meetings Joint

FAO/IAEA Division and EUCARPIA, Bari, Italy 1972). International Atomic Energy Agency, Vienna, pp. 303–343

Stadler LJ (1928a) Genetic effects of X-rays in maize. Proc Natl Acad Sci U S A 14(1):69–75

Stadler LJ (1928b) Mutations in barley induced by X-rays and radium. Science 68(1756):186–187

Stadler LJ (1929) Chromosome number and the mutation rate in Avena and Triticum. Proc Natl Acad Sci USA 15(12):876–881

Tester M, Langridge P (2010) Breeding technologies to increase crop production in a changing world. Science 327(5967):818–822

Till B, Jankowicz-Cieslak J, Huynh OA, Beshir MM, Laport RG, Hofinger BJ (2015) Low-cost methods for molecular characterization of mutant plants: tissue desiccation, DNA extraction and mutation discovery: protocols, 1 edn. Springer, Berlin

Till BJ, Zerr T, Comai L, Henikoff S (2006) A protocol for TILLING and Ecotilling in plants and animals. Nat Protoc 1(5):2465–2477

Tollenaar D (1934) Untersuchungen ueber Mutation bei Tabak. I Entstehungsweise und Wesen kuenstlich erzeugter Gen-Mutanten Genetica 16(1–2):111–152

Tollenaar D (1938) Untersuchungen ueber Mutation bei Tabak. II Einige kuenstlich erzeugte Chromosom-Mutanten Genetica 20(3–4):285–294

Tsai H, Howell T, Nitcher R, Missirian V, Watson B, Ngo KJ, Lieberman M, Fass J, Uauy C, Tran RK, Khan AA, Filkov V, Tai TH, Dubcovsky J, Comai L (2011) Discovery of rare mutations in populations: TILLING by sequencing. Plant Physiol 156(3):1257–1268

van Harten AM (ed) (1998) Mutation breeding. Theory and practical applications. Cambridge University Press, Cambridge

Weigel D, Mott R (2009) The 1001 Genomes Project for *Arabidopsis thaliana*. Genome Biol 10(5)

Yuan L, Dou Y, Kianian SF, Zhang C, Holding DR (2014) Deletion mutagenesis identifies a haploinsufficient role for gamma-zein in opaque2 endosperm modification. Plant Physiol 164 (1):119–130