

# Molecular nature of chemically and physically induced mutants in plants: a review

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## Abstract

More than 3200 new mutant varieties have been bred and used by millions of farmers, which has significantly contributed to world food security. A lot more mutants have also served as tools for gene discovery and functional analysis. Recent genomic approaches including TILLING (Targeting Induced Local Lesions In Genome) have enabled screening of mutations at the molecular level. This review describes the molecular nature of chemically and physically induced mutations and their repercussions. Analyses of TILLING reports indicate that chemically induced mutations are mostly nucleotide substitutions, but that mutation frequencies fluctuate among plant species ranging from one DNA lesion per 300 kbp in *Arabidopsis* to one DNA lesion per 30 kbp in bread wheat, which reciprocate with an increase in ploidy levels. The majority (>95%) of chemically induced DNA lesions are silent or missense mutations. Mutations induced by physical mutagens seem to be more diverse, including single-nucleotide substitutions, insertions, inversions and translocations, although short deletions (<15 bp) are relatively more predominant. The proportion of complex mutations (translocation, inversions, etc.) may increase with an increase in the linear energy transfer of radiations. In addition, the implications of these findings for the roles of induced mutants in breeding and gene function analysis are briefly discussed.

**Keywords:** gene discovery; mutagenesis; mutants; plant breeding

## Introduction

A great number of mutants have been generated and widely used in plant breeding programmes, which has successfully led to the release of more than 3200 new varieties in ~200 plant species (<http://mvgs.iaea.org/>). However, it was not until the beginning of this century that molecular genetic studies started shedding light on the nature of induced mutations. The integration of molecular and genomic techniques with induced mutagenesis, such as TILLING (Targeting Induced Local

Lesions In Genome), and their applications have already been critically reviewed in the past few years (McCallum *et al.*, 2000; Waugh *et al.*, 2006; Pathirana, 2011; Sikora *et al.*, 2011). The present review focuses on the molecular nature of mutants induced by various mutagens with the aim of helping researchers design better research strategies.

## Chemically induced mutations

Alkylating agents such as ethyl methanesulphonate (EMS), 1-methyl-1-nitrosourea and 1-ethyl-1-nitrosourea are the most commonly used chemical mutagens in plants (Leitao, 2012). EMS induces almost 100%

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GC > TA substitutions, while other mutagens generate >90% such mutations (McCallum *et al.*, 2000; Greene *et al.*, 2003; Cooper *et al.*, 2008; Sikora *et al.*, 2011).

TILLING studies have also enabled the estimation of mutation frequencies in different crops (Table 1). Higher mutation frequencies are achieved with increasing ploidy; for example, one mutation per 30 kb has been attained in hexaploid wheat and oats, while the maximum has been one mutation per 89 kb in diploid *Arabidopsis* (Table 1). Mutation frequencies can be increased by the optimization of treatment doses or selection of chemical mutagens, as has been shown for *Arabidopsis* and rice (Greene *et al.*, 2003; Till *et al.*, 2007; Suzuki *et al.*, 2008; Martín *et al.*, 2009). In practice, a higher mutation frequency may not always be effective because the proportion of non-synonymous mutations could become less as reported by Gottwald *et al.* (2009). Each report has presented a great variation in mutation frequencies among different genes; for example, Gottwald *et al.* (2009) reported mutation frequencies ranging from 1 per 870 kb for *Mlo9* to 1 per 200 kb for *HvHox1* in barley.

The number of mutations existing in a single  $M_2$  plant can be estimated based on genome size and mutation frequency. For example, a single *Arabidopsis*  $M_2$  plant may have 400 mutations, on average, while a bread wheat plant can have as many as 0.7 million mutations (Table 1). As most mutations are not desirable, it is essential that they be cleared before a mutant could be practically used as a new variety.

The majority of chemically induced mutations seem to be either silent (synonymous) or missense, with each accounting for slightly less than 50% (Fig. S1, available online). Nonsense mutations that produce knockout mutants are often less than 5% (Fig. S1, available online).

Most data on mutation profiles are obtained from seed-propagated crops. A recent TILLING study on banana has revealed that a high mutation frequency could also be achieved (Table 1). Of note is that up to 15% of the mutations were nonsense mutations (Fig. S1, available online) (Jankowicz-Cieslak *et al.*, 2012). It may reflect that less harmful mutations are eliminated during vegetative propagation than during seed propagation.

### Physically induced mutations

A number of physical mutagens have proven to be efficient for inducing mutations in plants (Mba *et al.*, 2012). Unlike chemical mutagens, physical radiations have quite different properties and can induce different types of mutations.

### $\gamma$ -Radiation

The most commonly used physical mutagen in plant breeding is  $\gamma$ -radiation. In rice, for example, more than 450 new varieties have been bred. By amplification and sequencing of target genes in induced mutants, Morita

**Table 1.** Mutation frequencies and estimated number of mutations in a single  $M_2$  plant derived from chemical mutagenesis

Plant species	Ploidy level	Genome size	Mutation frequency (1/kb) <sup>a</sup>	Mutations per mutant plant ( $\times 10^3$ ) <sup>b</sup>
<i>Arabidopsis</i>	2X	~120 Mb	1/300 <sup>c</sup> –1/89 <sup>d</sup>	~0.4–1.4
Rice	2X	~380 Mb	1/294 <sup>e</sup> –1/135 <sup>f</sup>	~1.3–3.0
<i>Triticum monococcum</i>	2X	~4.94 Gb	1/92 <sup>g</sup>	~54
Sunflower	2X	~5.0 Gb	1/480 <sup>h</sup>	~100
Barley	2X	~5.1 Gb	1/374 <sup>i</sup> ; 1/500 <sup>j</sup>	~100–140
Soybean	2X	~1.1 Gb	1/550–1/140 <sup>k</sup>	~20–80
Durum wheat	4X	~10 Gb	1/51 <sup>l</sup>	~200
<i>Brassica napus</i>	4X	~1.15 Gb	1/60–1/27 <sup>m</sup> ; 1/130.8–1/41.5 <sup>n</sup>	~9.2–28
Bread wheat	6X	~17 Gb	1/38 <sup>l</sup> ; 1/37.5–1/23.3 <sup>o</sup> ; 1/47 <sup>p</sup>	~440–700
Oats	6X	Unknown	1/40–1/20 <sup>q</sup>	–
Banana	3X	Unknown	1/47 <sup>r</sup>	–

<sup>a</sup> Mutation frequency is estimated as the number of mutations per kb of DNA sequence. In most cases, the average is quoted, e.g. 1/300 reported by Greene *et al.* (2003) and 1/89 reported by Martín (2009) for *Arabidopsis*; in a few cases, the range is given, e.g. 1/67–1/20 reported by Wang *et al.* (2008) in *Brassica napus* L. for information of variation. <sup>b</sup> The number of mutations in a single plant is estimated by genome size  $\times$  mutation frequency. <sup>c</sup> Greene *et al.* (2003). <sup>d</sup> Martín *et al.* (2009). <sup>e</sup> Till *et al.* (2007). <sup>f</sup> Suzuki *et al.* (2008). <sup>g</sup> Rawat *et al.* (2012). <sup>h</sup> Kumar *et al.* (2013). <sup>i</sup> Talamè *et al.* (2008). <sup>j</sup> Gottwald *et al.* (2009). <sup>k</sup> Cooper *et al.* (2008). <sup>l</sup> Uauy *et al.* (2009). <sup>m</sup> Wang *et al.* (2008). <sup>n</sup> Harloff *et al.* (2012). <sup>o</sup> Dong *et al.* (2009). <sup>p</sup> Chen *et al.* (2013). <sup>q</sup> Chawade *et al.* (2010). <sup>r</sup> Jankowicz-Cieslak *et al.* (2012).

*et al.* (2009) identified 22 mutations. In addition, individual forward genetics studies have also identified dozen more mutations. Altogether, a total of 66  $\gamma$ -ray-induced mutations with the nature of DNA lesions are summarized in Table S1 (available online).

### Fast neutrons (FNs)

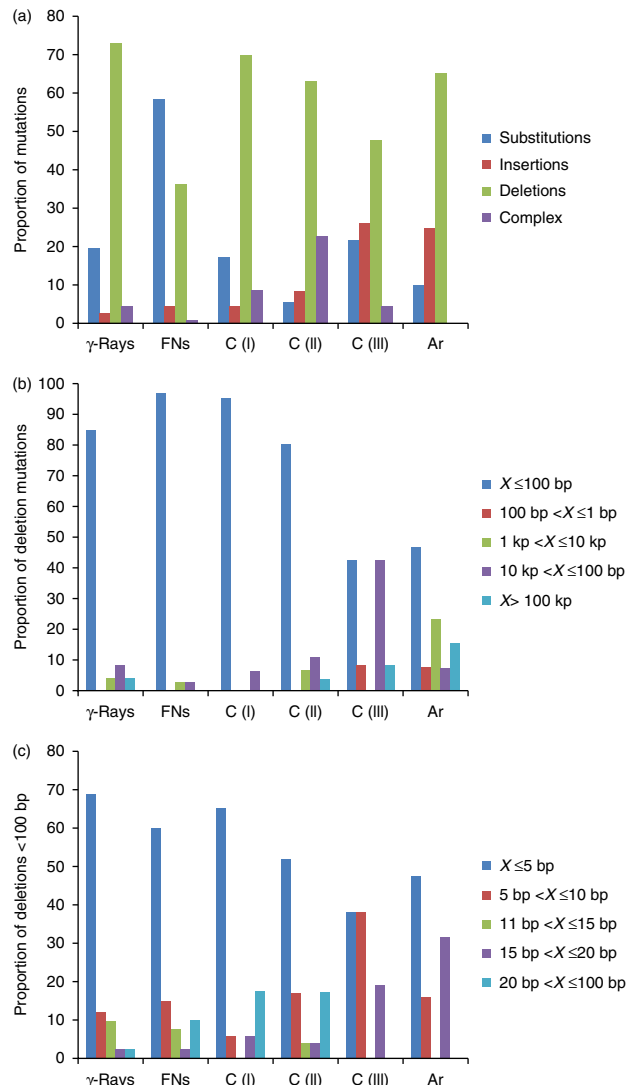
FNs have long been used for both forward and reverse genetic studies in plants. The identification of a  $\sim$ 460 kb FN-induced deletion enabled the cloning of a key gene involved in nodulation in legume crops (Men *et al.*, 2002; Searle *et al.*, 2003). In *Arabidopsis*, a survey of FN-induced mutations indicated that the deletion size ranged from 5 to 35 kb (Li and Zhang, 2002). Using the reverse genetics approaches such as ‘Deleteagene’ (Li *et al.*, 2001) and De-TILLING (Rogers *et al.*, 2009), mutations with deletions of 0.8–12 kb in *Arabidopsis* and rice and of 0.42–1.72 kb in *Medicago truncatula* have been identified. Although these early studies have suggested that FNs induce large deletions, a recent study has demonstrated that single-base substitutions and other types of mutations also exist (Belfield *et al.*, 2012).

### Ion beam radiations (IBRs)

IBRs differ from  $\gamma$ -rays in their linear energy transfer (LET). Different LETs could be achieved by adjusting the type of ions and their energy (Abe *et al.*, 2012); for example, carbon ions ( $^{12}\text{C}^{6+}$ ) can have different LETs (Fig. 1). IBRs seem to produce more large deletions ( $>1$  kbp) and complex mutations than single-base substitutions and short deletions. Deletions up to 225 kbp, inversions of fragments of  $\sim$ 3.4 Mbp and (reciprocal) translocations have been reported in *Arabidopsis* mutagenized using carbon ion beams (Shikazono *et al.*, 2005).

### Comparison of mutation profiles

Analysis of the above-mentioned mutations indicated that all radiations could produce deletions and other types of DNA lesions, though the former predominate in most cases (Fig. 1(a)). Base substitutions, previously assumed to be rare, indeed represent the majority in FN-induced mutations (58.2%) (Fig. 1(a)) as revealed by whole-genome resequencing (Belfield *et al.*, 2012). The size of deletions ranges from 1 bp to half million bp (Fig. 1(b)), with short ones ( $<5$  bp) predominating in all cases (Fig. 1(c)). In addition, insertions up to



**Fig. 1.** Types and profiles of mutations induced by  $\gamma$ -rays in rice and fast neutrons (FNs) and ion beams in *Arabidopsis*. C (I), C (II) and C (III) represent carbon ion ( $^{12}\text{C}^{6+}$ ) beams with linear energy transfer (LET) of 22.5–30.0, 113 and 290  $\text{keV}/\mu\text{m}$ , respectively. Ar represents argon ion ( $^{40}\text{Ar}^{17+}$ ) beam with a LET of 290  $\text{keV}/\mu\text{m}$ . Data on  $\gamma$ -rays were extracted from Table S1 (available online) with a reference list; data on FNs were extracted from Belfield *et al.* (2012) and those on C (I) from Kazama *et al.* (2011), on C (II) from Shikazono *et al.* (2005) and on C (III) and Ar from Hirano *et al.* (2012).

7 kbp have also been reported even in  $\gamma$ -ray-induced mutants (Chen *et al.*, 2013; Zhao *et al.*, 2013). Complex mutations, such as inversions and translocations, which are often combined with deletions or insertions (Hirano *et al.*, 2012), have more frequently been identified in ion beam-mutagenized plants (Fig. 1(a)). Furthermore, carbon ions with greater LETs seem to produce more large-sized deletions/complex mutations (Hirano *et al.*, 2012).

## Perspectives

Substantial progress in molecular elucidation of mutations induced by various mutagens during the past decade has immediate implications for the selection of a proper mutagen for a particular research purpose. For example, IBRs should be a nice choice for deleting clustered genes, while chemical mutagens are better suited to induce missense mutations, which might be gain-of-function mutations, e.g. tolerance to herbicides. The dense background mutations indicate, on the other hand, that it would take more generations than previously taught in textbooks to develop a stable mutant line. No whole-genome analysis has been carried out for plants mutagenized by any mutagen except FNs; therefore, we have to be cautious while interpreting the differences in mutation profiles. In this regard, genome resequencing studies should also be carried out for obtaining holistic mutation profiles in plants mutagenized using chemical mutagens as well as  $\gamma$ -rays and IBRs.

## Supplementary material

To view supplementary material for this article, please visit <http://dx.doi.org/10.1017/S1479262114000318>

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