Plant Molecular Mutation Breeding

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Abstract

The advance in molecular genetics and DNA technologies has brought plant breeding including mutation breeding into a molecular era. Plant molecular mutation breeding is here defined as mutation breeding in which molecular or genomic information and tools is used in the development of breeding strategies and in the implementation of the breeding process. It is built upon the science of DNA damage, repair and mutagenesis, plant molecular genetics and genomics of important agronomic traits as well as induced mutations. Mutagenic treatment, super-mutable genetic lines, molecular markers and high throughput DNA technologies for mutation screening such as TILLING (Targeting Induced Limited Lesions IN Genomes) are the key techniques and resources in molecular mutation breeding. Molecular mutation breeding will significantly increase both the efficiency and efficacy of mutation techniques in crop breeding. A perspective molecular mutation breeding scheme is proposed for discussion.

Media summary

Fast growing knowledge of plant molecular biology and genomics-based DNA technologies will transform plant mutation breeding into a new paradigm.

Key Words

Mutagenesis, genomics, mutation techniques, molecular mutation breeding

Introduction

Plant breeding is often regarded as applied genetics, and so is mutation breeding. One of the most important breakthroughs in the history of genetics was the discovery of experimental mutagenesis in the early 20 century, which later brought about plant mutation breeding. Without knowing much of the molecular biological basis, a vast amount of genetic variability was induced in most economically important plant species, and a small portion of those induced variations has resulted in the development of more than 3000 mutant varieties worldwide in about 180 plant species during the past 60 years (Shu and Lagoda 2007). During the past decade, with the unprecedented development in plant molecular genetics and functional genomics, scientific exploration on induced mutation in plants has progressed dramatically from basic research on mutagenesis in plants to the development of advanced genomics-based technologies to their unique applications in gene discovery and development of novel crop traits (Waugh et al. 2006). These developments are bringing plant mutation breeding into a new paradigm – Plant Molecular Mutation Breeding.

Genetics and features of classical mutation breeding

Plant breeding methods in principle can be classified into three systems: recombination breeding, mutation breeding and transgenic breeding, each with unique way of generating variation and of selecting target lines (Table 1). In terms of mutation breeding, the generation of new mutated alleles is the core and most unique feature. The genetics behind mutation breeding include differences in the sensitivity of different genotypes and plant tissues to different mutagens, which is often measured using lethal doses (LD); genetic chimeras after mutagenic treatment and its effect on transmission of mutated alleles and segregation in the followed generation; also known is the often recessive nature of induced mutations. Such knowledge of genetics is important for

establishing proper doses and modes of mutagenic treatment, and also for the methodology of harvesting and growing M_2 populations (Table 1).

Table 1 Genetics of three unique breeding methods for seed crops

	Recombinant breeding	Mutation breeding	Transgenic breeding
1. Source of genetic variation	Recombination of gene alleles from parental varieties.	New alleles artificially and randomly created from endogenous genes.	Insertion of new genes or modification of endogenous genes.
2. Transmission, expression and inheritance	No selective transmission; co-segregation of closely linked alleles.	Induced mutations subject to diplontic and haplontic selection.	Expression of transgenes subject to position effect or silencing.
3. Nature of gene action	Dominant, recessive alleles, and QTLs.	Mostly recessive alleles.	Mostly dominant alleles.
4. Breeding generations	About 10 generations.	About 2-3 generations.	About 3 generations.

Mutation breeding has its own advantages and limitations. The advantages include creation of new gene alleles that do not exist in germplasm pools; induction of new gene alleles for a commercial variety so new varieties carrying desired mutation alleles can be directly use as a commercial variety. The limited genetic changes of any single plant of a mutated population and the often recessive nature enable breeders to develop a new variety in a short breeding cycle. The disadvantage of mutation breeding is its limited power in generating dominant alleles that might be desired; it is also less effective than cross breeding for a trait needs a combination of multiple alleles, such as tolerance to abiotic stresses. The low mutation frequency requires growing and screening a large population for selection of desired mutants at a reasonable confidence. This becomes very expensive for traits that have to be evaluated through laborious phenotypic analysis.

Molecular genetics and genomics related to mutation breeding

The rapid development of plant molecular genetics and genomics in areas relevant to mutation breeding has been reinvigorating this breeding method; it is expected that mutation breeding will directly benefit from the rapid scientific and technological advances in molecular genetics and genomics.

DNA damage and repair

It has been well documented that DNA is subject to continuous damage and the cell has an arsenal of ways of responding to such injury; although mutations or deficiencies in repair can have catastrophic consequences for organisms, mutations are nonetheless fundamental to life and evolution (Friedberg 2003). With the accumulating knowledge of the molecular genetics of DNA damage and repair, we can now elucidate many of the phenomena that we have observed in classical mutagenesis, e.g. the differences of sensitivity to different mutagens among plant species and among plant materials. There are different pathways for the repair of DNA damages caused by different types of mutagen, for example, gamma irradiation often leads to DNA double strand breaks (DSBs, Puchta 2005), ultraviolet (UV) radiation results in covalent dimerization of adjacent pyrimidines (Friedberg 2003), while chemical mutagens cause mis-pairing or nucleotide excision. These knowledge is very important for properly designing mutagenic experiment in a way that an enhanced mutation frequency could be achieved. For example, there are two pathways in DSB repair: homologous recombination (HR) and non-homologous end-joining (NHEJ; also known as illegitimate recombination). HR repair is quite precise and results in few mutations, while NHEJ is an error –prone process thus can produce mutations (Puchta 2005). Therefore, a genetic line defect in HR repair, or haploid materials such as pollen or anther (lack of homozygous DNA template for HR) is expected to produce a high frequency of mutations after radiation

treatment (the transgenes can be separated from the mutations and the mutant lines will become stable. Such knowledge may also provide clues to identify new chemicals that can induce mutations in plants while having limited toxicity to humans.

Molecular genetics of induced mutations

Cells with damaged DNA will survive only when these damages are repaired either correctly or erroneously; the result of erroneous repairs will be fixed in the genome as induced mutations. The nature of DNA damage caused by different types of mutagen to a great extent determines the molecular feature of induced mutations. For example, chemical mutagen EMS often lead to mutations of G/C to A/T transition (Till et al. 2007), while ion beam implantation could cause deletion of f DNA fragment of various size (Naito et. 2005). Although information is limited so far in this field, such knowledge will definitely help choose proper mutagen for different purposes in mutation breeding. For example, DNA deletions in most cases will cause recessive mutations, while nucleotide substitution may produce a dominant allele. Therefore, when a recessive mutation could solve the problem, irradiation might be a better choice, while when a dominant mutation is needed (for example for herbicide resistance); a chemical mutagen might be more useful. It is also important for establishing proper methods of DNA based mutation screening.

Molecular genetics of target trait

Plant molecular breeding in general depends on the understanding of the molecular genetic control of target traits of interest. Molecular genetic information is also of great help in developing a proper mutation breeding strategy. First, it is important for assessing the feasibility and potential to induce a mutation of interest. Since the mutation frequency for any given fragment of DNA or gene is more or less similar, therefore, the opportunity to obtain a mutant of different traits would be dependant on the number of genes that control the trait. For example, many genes can affect the growth duration (i.e. days from sowing to heading for cereals), therefore the mutation frequency of such trait is often far higher than single-gene controlled traits (Fu et al. 2008). Second, a mutation may have pleiotropic effects if the gene is at the upstream or at the middle of a long biosynthetic pathway, such as the MIPS gene in phytic acid biosynthesis precautions should be made for such a mutation project. Third, knowledge of genes controlling a trait of interest would constitute the very basis of the TILLING (Targeting Induced Limited Lesions IN Genomes) method.

Perspective of molecular mutation breeding

With more knowledge of DNA damage, repair and mutagenesis becoming available, more traits of interest being dissected at the molecular genetics level, and more molecular techniques developed and commanded by breeders, mutation breeding will be transformed into a new paradigm. A perspective scheme is proposed with the potential to significantly enhance the efficiency of plant mutation breeding (Figure 1).

Effective mutation induction

Mutation induction is the starting step in mutation breeding, and its low frequency has been a severe limiting factor. Equipped with knowledge of the DNA damage and repair, we should be able to design strategies of mutagenic treatment to significantly increase the mutation frequency. For example, smartly combined use of chemical and physical mutagens, recurrent mutagenic treatments, would increase mutation frequency, since theoretically each time of treatment would cause DNA damages which should be repaired after treatment, and consequently introduce new mutations each time. We should be able to select a suitable mutagen and starting material for specific purpose, for example, chemical mutagens should be more suitable for inducing dominant alleles while physical mutagens might be better used for recessive mutations. We should also be able to produce super mutable plant lines by genetic modification of specific genes in DNA repair system as shown by Leonard et al. (2003). Similarly, suppression of the HR system could enhance the NHEJ repair and hence a higher mutation frequency when treated with physical radiations.

High throughput mutation screening

Mutation screening has been another bottleneck in mutation breeding, particularly for traits that can't be visually identified and has to be assessed by costly or laboriously chemical test. This is being changed due to the establishment of DNA-based mutation screening techniques during the past few years (Waugh et al. 2006). The TILLING system (Colbert et al. 2001), for example, based on the CEL I cleavage of mismatches, has been exploited in several plant species with success (Waugh et al. 2006). A variety of modified version of TILLING has already become available, such as that for detection of deletions --de-TILLING. It is foreseen that mutation screening technology will become more, high throughput, powerful and affordable with rapid development of DNA technologies including high throughput DNA sequencing techniques.

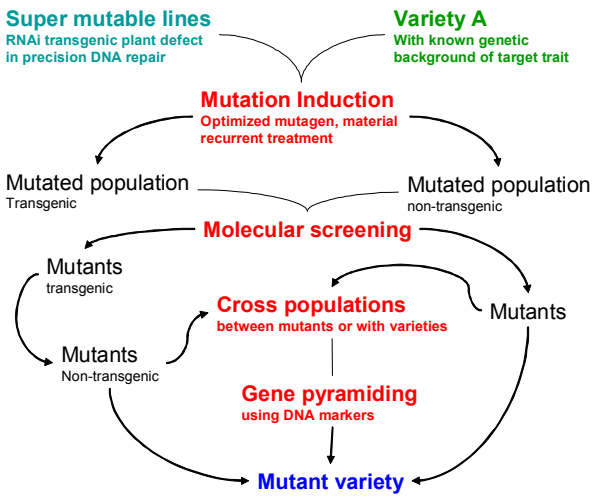

Figure 1. Plant molecular mutation breeding scheme

Mutation utilization

In classical mutation breeding, induced mutations are embedded in mutants that are either directly or indirectly (through crosses with other varieties) used for developing a new variety, which is rather difficult to trace the mutated genes in subsequent breeding. It is now possible to tag mutated genes, pyramid them into a single elite breeding line, and follow up in subsequent breeding programs.

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