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REVIEW ARTICLE

Mutant Resources and Mutagenomics in crop plants

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ABSTRACT

Agricultural sustainability and food security are major challenges facing continued population growth. Integration of existing and new technologies for the induction and exploitation of genetic diversity towards developing healthier, nutritious and productive crops is the need of the hour. Mutagenesis is a proven technology for the development of improved or novel varieties with desirable traits. Several mutant genes have been successfully explored, either directly or indirectly, to complement crop productivity. The advent of genomics approaches and plant genome sequencing has benefitted mutation discovery and mutant characterization. Plant mutant repositories are being established to serve as platforms for basic and applied research in crop improvement. This review briefly outlines the impact and molecular/genomic characterization of induced mutations in crop improvement.

Keywords: Induced mutations; Mutants; Mutagenomics; Plants

INTRODUCTION

Agricultural sustainability has become the main concern in the context of depleting arable land and water resources and climate change driven environmental extremities. It is essential that our agricultural research will have to be geared up to meet the global food demand for feeding increasing human population (UNFPA, 2012). Food security can be achieved through breeding approaches by improving environmental stress tolerance, water use efficiency, pathogen resistance and nutritionally enriched foods and production of high-value bioactive compounds. Agricultural innovation has always dealt with science-based products and processes which have contributed to improved productivity and sustainability (Pretty, 2008). In this context, identification of the most appropriate technologies and developing of a knowledge base of agricultural crops has become a priority. Consequently, the demand for agricultural crops for their use as food, feed, fuel and energy has increased over the years and there is a need to adopt innovative technologies of agricultural sustainability.

Induction and exploitation of genetic diversity is an established genetic approach in crop improvement. Plant breeding techniques, mutagenesis, biotechnology, genetic engineering and molecular breeding have played a pivotal role in exploiting available germplasm resources for developing improved cultivars (Lusser et al., 2012; Hallerman and Grabau, 2016). Transgenic approaches have been adopted in several crops but the technology is still to reach the developing countries since it requires skilled scientific personnel, well developed laboratory set up with high end equipment, available resource of isolated genes and promoters, compliance with biosafety practices, crop production system and consumer acceptance (Parry et al., 2009; Jain, 2010; Suprasanna et al., 2017). In this regard, mutagenesis offers as a simple and effective means of inducing genetic variation. A single induced mutant can have several desirable traits, e.g. disease resistance, high yield, quality, plant architecture, and abiotic stress tolerance. This is in contrast to transgenic plants where in single gene trait is often expressed. The spontaneous mutation rate is too low (10^{-5} – 10^{-8}) and is inadequate to be utilized for enhancing genetic variability in crop breeding. Induced mutations with physical and chemical mutagen treatment enhances rate of mutation rate enabling mutant lines to use in plant breeding programs, especially in those crops with limited genetic variability (Jain, 2005; Jain and Suprasanna, 2011). The constraints in case of conventional breeding such as low vigour, narrow gene pool, complex genomes, reduced fertility and the lengthy breeding/selection cycle

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collectively impede plant improvement can be addressed through induced mutagenesis (Suprasanna et al., 2012). As per the FAO/IAEA Mutant Varieties Database, more than 3200 mutant varieties are released for cultivation in different countries (Suprasanna et al., 2014). Mutants are usually produced using radiation, chemicals, T-DNA or transposons (Bradshaw, 2016). Use of physical mutagens has yielded the majority of mutant varieties among which gamma rays have been mostly used followed by other radiation methods (Suprasanna et al., 2014).

In the past 5-6 decades, application of induced mutations has been on the forefront of developing and developed countries in producing several superior crop varieties, and that has made a greater economic impact on food production and feeding people (Kharkwal and Shu, 2009). The leading countries having the highest number of officially released, mutant varieties are China, India, the former USSR, The Netherlands, Japan and USA. Mutagen wise, higher proportion (>50%) of mutants has been developed by using gamma rays as compared to other mutagens. Crop wise, maximum mutants were developed in cereals followed by ornamentals, legumes and pulses and other crops including vegetables, forage, edible oil plants and tree species (Mba, 2010). Among cereals and all other crops, higher number of mutants were developed in rice (700 mutant varieties) followed by barley, wheat, maize, durum wheat, oat, millet, sorghum and rye (Suprasanna et al., 2015). As per the FAO/IAEA database, 1,825 mutants (accounting to 57%) have either better agronomic and botanical traits; of these, 577 mutants (18%) are developed for increase in yield and related traits, 321 mutants (10%) for better quality and nutritional content, 200 mutants (6%) for biotic and 125 mutants (4%) for abiotic stress tolerance (Suprasanna et al., 2015). These mutant varieties have made a greater economic impact contributing to millions of dollars annually to local economies (Ahloowalia et al., 2004; Jain, 2005).

The mutagenesis research has been advanced by genomics advances including methods to detect genetic variation, select mutant phenotypes, TILLING (Targeted Induced Local Lesions IN Genomes), ecoTILLING, resequencing, RNAi (RNA interference), mismatch site-specific mutagenesis, homoeologous recombination, forward and reverse genetics via transposable elements, gene replacement, gene addition, and transcriptome modification by mutagenic treatment, aneuploidy, and uniparental chromosome loss (Phillips and Rines, 2009). The induced mutations cover a variety of genome modifications of a number of genes which include Single-nucleotide polymorphisms (SNPs) and small insertions and deletions (indels), chromosomal rearrangements, duplication of genes and transposable element mediated insertion/deletion events (Nogue et al., 2016; Negi et al. 2016). Understanding of the mutant gene structure, functions, spatial and temporal expression and genetic regulation can be useful for improving agronomically-important crop plants. In this article, we present an overview of mutants as means of novel genetic resource and for analyzing gene function.

Developing 'mutant' resources

Mutants are usually produced using physical, chemical and biological agents (Bradshaw, 2016). Gamma irradiation results in small deletions (1-10 bp) while neutrons cause 300 bp to 12 kbp deletions and chemical mutagens result in point mutations mainly G/C-to-A/T transitions (Morita et al., 2009). On the other hand, ion beams have high linear energy transfer (LET) ranging from 22.5 keV μm^{-1} to 4000 keV μm^{-1} compared to 0.2 to 2 keV μm^{1} LET of γ-rays and X-rays (Ryuto et al., 2008). Heavy-ion beam (HIB) irradiation is shown to be superior for mutation breeding as higher rate of mutations can be obtained at low doses (Hirano et al., 2015). It is also observed that HIB induces more localized, dense ionization and causes direct damage to DNA. Compared to X-rays or gamma rays, heavy ion-beams can be used to alter single characteristic and thus new cultivars can be developed which will have selected target trait while not disturbing the existing characteristics of the parent cultivar.

Ion beam irradiation research has been widely studied and great number of mutant varieties have been developed in China and Japan (Nakagawa, 2009; Wu et al., 2005). High-energy ion beams are used routinely for creating variation in ornamental plants of high market value in Japan, whereas, low energy ion beam research in China is focused on agriculturally important crops. Several salt-tolerant mutants of rice, vegetables like spinach, fruits like muskmelon, citrus fruits tree, coniferous tree, etc., have also been ion-beam irradiated to produce new varieties. Tanaka et al. (2010) outlined the success from ion beam mutagenesis in ornamentals. The first ion-beam-induced varieties were from *Verbena sp.*, carnation (*Dianthus caryophyllus)*, and Chrysanthemum (*Dendranthema grandiflora*). This was followed by many varieties of *Petunia hybrida*, *Torenia, Dahlia, Osteospermum* with new flower color and shape. In chrysanthemum 'Aladdin' and 'Aladdin 2' with a few axillary flower bud, were developed and commercialized successfully. By using carbon ions, a cultivar 'KNOX' of *Ficus thunbergii* was developed with better assimilation ability to fix atmospheric nitrogen. Carbon ion beam has also resulted in the development of a new variety of cherry blossom tree (Nishina Otome) which can bloom in all the seasons. In addition to ion beam, space mutagenesis has also been exploited for mutation induction. More than 60 new crop varieties with improved yield, quality and multiple stress tolerance have been released in China (Liu et al., 2007).

Plant mutagenesis research has components of, i) development of induced mutants for morphological, biochemical and physiological traits, and ii) study of their genetic stability and agronomical performance. In this context, plant mutant libraries, generated by chemical or physical mutagenesis, will have to be developed and maintained as mutant genetic resource centres. Such mutant resource offers as an excellent material for understanding the radiation damage, physiological basis, ultrastructural changes and plant metabolic pathways, besides for gene mapping and is valuable for functional genomics (Lundquist, 2009; Mirajkar et al., 2016). Both functional genomics and mutant gene detection studies will require a mutant library having a high mutation density (Tsuda et al., 2015). For example, TOMATOMA (http://tomatoma.nbrp.jp/) is a tomato mutant database offers phenotypic data of ethylmethane sulfonate (EMS) γ-ray irradiation derived tomato mutant lines (Shikata et al., 2015). In wheat, Gu et al. (2017) developed a EMS based-mutant resource with high frequency phenotypic and genotypic variation for plant traits. Recently, 1,504 FN derived rice mutant lines were whole-genome sequenced and this resulted in the identification of 91,513 mutations affecting 32,307 genes (Li et al. 2017). This study has established a WGS rice mutant collection as an open access resource called KitBase which integrates multiple bioinformatics tools and enables users to search the mutant collection, visualize mutations, download genome sequences for functional analysis.

Muta-genomics tools

Mutational genomics is becoming an important tool to investigate the mutational events orchestrating genetic modification in mutant traits. Such mutational events can be characterized globally by using high throughput genomics technologies such as cDNA-amplified fragment length polymorphism (cDNA, AFLP), single strand conformational polymorphism (SSCP), serial analysis of gene expression (SAGE), microarray, differential display, TILLING, high resolution melt (HRM) analysis (Nadeau, 2000). HRM technology has been employed to detect mutations and induced variability in tomato, wheat, maize, and sugarcane (Gady et al., 2009; Dong et al., 2009; Li et al., 2010). Study of the molecular basis of induced mutations is an essential aspect in selecting which mutation induction technique will be appropriate for analyzing gene function (Nawaz and Shu, 2014). Several mutagenesis methods such as ethyl methanesulphonate, T-DNA insertion, transposon tagging and ionizing radiation have provided key information on the nature of mutations. While EMS based chemical mutagenesis mostly results in point mutations, the T-DNA insertional mutagenesis or transposons often disorder the gene sequence. On the other hand, ionizing radiation induces deletions resulting in a high fraction of knock-out mutations (Sato et al., 2006). The size of the deletion can also be pre-selected using proper LET levels of heavy-ion irradiation (Kazama et al., 2011).

Both the approaches of forward and reverse genetics are integral to mutagenesis as mutants will be essentially required for gene function analysis (Fig. 1). The strategy of reverse-genetics using induced mutations, viz. TILLING method is a high throughput tool. It has been used in most plant species for screening mutations in mutant populations generated with chemical mutagens, such as EMS, however the technique can also be adopted for use with mutant progeny developed through gamma and fast neutron irradiation (Till et al., 2007; Jain et al., 2010). For example, the De-TILLING technique could be very well

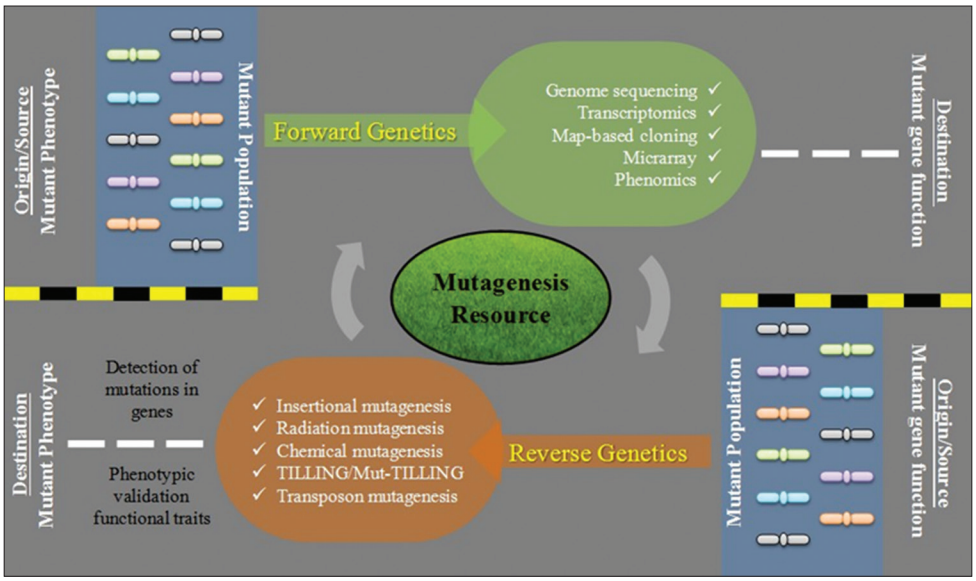

Fig 1. A roadmap of forward and reverse genetic approaches of plant mutagenomics

used to identify a specific mutant in a pool of 6,000 lines. TILLING ensures detection of mutations within genes of interest and to associate such mutations to a definite phenotype, for which gene(s) linked to the phenotype and the gene sequence is known (Sikora et al., 2011). MutMap is a recent development for cloning EMS-induced alleles in rice using a bulked segregation strategy, and the method is further extended to enable alleles to be cloned without outcrossing (Abe et al., 2012; Fekih et al., 2013). Wilde et al. (2012) presented an overview of screening in horticultural crops for natural or induced allelic diversity in over 100 candidate genes for traits of commercial interest, such as longer shelf-life (tomato, melon), improved starch quality (potato), and virus-resistance (peppers, tomato). An extension of the TILLING technique is 'TILLING by Sequencing' (TbyS) which relies on high throughput next-generation sequencing to speed up TILLING workflow (Tsai et al., 2011). Kumar et al. (2017) have outlined the advantages of the TbyS methodologies applied to identify point mutations from mutagenised populations. Several bioinformatics methods and databases (CODDLE for prognosis of most suitable gene regions for TILLING analysis; SIFT (Sorting Intolerant from Tolerant) and I-Mutant3.0 for Prediction of mutation effect on protein stability) are now available for monitoring of mutation and checking of mutation effect on protein stability and function (Slota et al., 2017).

High-throughput DNA sequencing methods such as next-generation sequencing, exon capture method are now available for mutation detection in a more efficient and cost-effective mode (King et al., 2015). Henry et al. (2014) analyzed mutations in EMS-derived mutant progenies of rice and wheat by using multiplexed global exome capture and sequencing coupled with bioinformatics tools and detected ~18,000 induced mutations. In EMS induced and gamma ray induced mutants of tomato, whole-genome shotgun sequencing analysis was used to calculate the spectrum and distribution of DNA mutations at genome level in the Micro-Tom genome (Shirasawa et al., 2016). The authors found that major mutations in the EMS mutants were C/G to T/A transitions type, while in the gamma-ray mutants, mutations were C/G to T/A transitions, A/T to T/A transversions and A/T to G/C transitions. In case of fast neutron irradiation, NGS analysis of mutants indicated higher incidence of single base substitutions than deletion mutations, and of small deletions (<10 bp) than large deletions in Arabidopsis (Belfield et al., 2012) and *Phaseolus vulgaris* (O'Rourke et al., 2013). These studies suggest that NGS method can be used to illustrate the heavy-ion-induced mutations to determine the comprehensive nature of induced mutations at the whole-genome level. Sometimes, precise detection of causal mutations in a polymorphic background is a challenge. Yan et al., (2016) have described an NGS-based method, SIMM (Simultaneous Identification of Multiple Causal Mutations) to study multiple rice mutants and identified seven new mutant alleles which were later confirmed by phenotype association method.

Identification of novel traits of interest in mutated populations can be done by different biochemical and physiological screening methods (Sikora et al., 2011). However large scale mutation induction studies will require high throughput phenotyping tools. This becomes highly demanding as researchers generate hundreds of induced mutations with different phenotypic effects and often, a majority of them are discarded possibly due to the lack of appropriate phenotypic screens (Nadeau, 2000). It is thus necessary to devise phenotypic assays for traits that have a genetic basis and thus, mutations affecting the trait can be discovered. In order to increase the scope of screening phenotypes from a large collection of mutants, high throughput phenotype screening and phenomics platforms are developed based on imaging and image processing (Rahaman et al., 2015).

Precise genetic modification (mutagenesis)

Aforementioned account reiterates the need and development of genetic resource of novel genetic variation to be introduced into cultivated varieties while taking advantage of existing natural genetic variation or through induction of mutations. This has taken a step further with the advent of new genetic tools of genome engineering for precise genetic manipulation of DNA in living cells (Voytas and Gao, 2014) to create new or improved traits. This genetic modification is often achieved by harnessing the pathways of DNA repair and the arsenal includes sequence-specific nucleases (SSNs) for the repair of DNA double-strand-breaks (DSBs). In the past few years, advancements in genome engineering has adopted different SSNs, for ex. zinc finger nucleases (ZFNs), engineered homing endonucleases or meganucleases, transcription activator-like effector nucleases (TALENs), and clustered regularly interspersed short palindromic repeats (CRISPR)/Cas9 reagents (Voytas, 2013). Lawrenson et al. (2015) induced mutations by using RNA-guided Cas9 in target genes (ABA-inducible plasma membrane protein and GA4 orthologues) in barley and *Brassica oleracea* and showed stable transmission of these mutations. Another example is on reduction in the phytate content in maize seeds since it limits mineral absorption. Shukla et al. (2009) demonstrated that use of tailored ZFNs in maize gene encoding inositol-1,3,4,5,6-pentakisphosphate 2-kinase (IPK1) could alter the profile of the inositol phosphate. Homologous recombination based site-directed mutagenesis has also been suggested as the precise mutation induction method for targeting specific genes (Saika et al. 2011). Specific

mutations were induced in OASA2 which is a subunit of anthranilate synthase, an enzyme involved in biosynthesis of tryptophan in rice and achieved 230-fold higher tryptophan levels. Song et al., (2016) reviewed applications of crop genome editing using CRISPR/Cas9 for mutation induction in several crops and noted mutation rates of 33% in sorghum, 13.1% in maize, 3.9% in citrus, 51.7% in poplar, 59 - 76% in soybean and 5.6% in wheat. Sun et al. (2017) studied targeted mutagenesis in starch branching enzymes (SBEI and SBEIIb) in rice using CRISPR/Cas9. While the mutation frequencies ranged from 26.7 to 40%, sbeII mutants showed significantly increased amylose content and resistant starch content with structural changes and nutritional characteristics. This study suggests that application of CRISPR/Cas9 technology can be employed to develop a high amylase rice mutant.

CONCLUSIONS

Mutation breeding has greater impact in sustainable crop production by developing new mutant varieties. With the advances in genomics research and availability of genome sequences, induced mutants continue to be a genetic resource for elucidating genetic mechanisms and metabolic pathways. Genomics research on the molecular nature of mutations could be useful in selecting the appropriate mutation induction techniques (e.g. ion beam) for gene function analysis. Muta-genomics tools enable understanding of mutational events towards genetic modification of mutant traits. Genome sequencing has made it possible for mutational events to be characterized globally by using high throughput genomics platforms. The development and maintenance of plant mutant repositories could offer as excellent platform for basic and applied research in crop improvement besides for gene mapping and functional genomics based research.

Authors' contribution

Both the authors contributed to the overall framework and writing of the paper.

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