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Mutation Breeding: Is it supplementing the Genetic Erosion?

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Abstract

Genetic variation in crop plants is one of the major resources, which helps breeder to select desired plants and solve crop issues. Mutation Breeding is one of the main approaches used in agricultural research to induce genetic variation. It is widely used to improve the genetics of crop where natural variation is absent. Genetic variability can be introduced into field crops like wheat, maize, rice, pulses etc. with the help of mutagenic agents. Recent technological outbreaks widened the scope of this field. It enables the researcher to work with non-conventional crops with limited resources and utilize this technique in crop improvement programs.

Keywords: Mutagenesis, Genetic Variation, Field crops and Mutation Techniques.

Introduction

A mutation can be defined as the immediate genetic change that is inheritable. This genetic change occurs in DNA, but the source is not as always recombination or crossing over (Pathirana, 2011). This genetic change can be natural and induced artificially. This mutation can be useful for mankind because it provides a variation that is heritable. In plant breeding it can be used as an important tool for creating the genetic variants and using them for improving the crop plants. Mutation can provide quick variation and hence it is the source of evolution for them. In comparison to conventional breeding where variation is produced by crossing two different genotypes and usually the genotype having favorable traits is poor in all other parameters. It requires back crossing with the stronger parents again and again to fix all the genes. They also have poor combining abilities, an important example is different aromatic and non-aromatic cultivars of rice (Bourgis et al., 2008; Pathirana, et al., 2009). Mutation can play an important role in developing traits of interest and utilizing them. Linkage is also trouble for the case when useful genes are linked to undesirable ones. Mutation can separate these linked genes or can produce independent variations. Some plants species like bananas are unable to produce seeds so in these plants, mutation is vital for producing variability (Chai et al 2004; Medina, et al., 2011). Mutation is an important source of variation in vegetatively propagated plants where crossing is uncertain. In vegetatively reproduced plants, mutation is also an important source of variation because many limitations are there in crossing these plants (Datta,2009; Kondo et al, 2009).

Variations are prerequisites to evolution, so breeding depends on genetic variation. All the variation present in the nature is not useful for breeding because some of variation is due to cross combinations or environmental interference (Pathirana, 2011). In history green plants have been the vital component of the human diet as in ancient times human population consisted of hunters and they depended solely on green vegetation and hunting for their food. Population growth increased the food demand and they shifted towards a safer food system i.e. cultivation of food crops. The better plants were selected and used further. This selection was the start of plant breeding.

After the discovery of Mendel's work genetics gained importance in plant breeding. The laws of hereditary were discovered and utilized in understanding genetics. Later on, hybridization revolutionized the plant breeding because it enabled the accumulation of desirable genes in one individual that is superior to both of its parents (Pathirana, 2011). The use of X-rays for inducing variations in genes was started by Lewis John Stadler in 1920 and introduced mutation breeding (Shu et al., 2012). The variation created in such a way is further used by genetic recombination. Independent assortment of alleles makes different combinations in plants as well as animals. These variants are subjected to natural selection leading to evolution. The frequency of natural or spontaneous mutations to occur is very less and hence there is limited use in

plant breeding (Pathirana, 2011). Mutation can produce both positive and negative effects in plants. Now a day, mutations are induced artificially through artificial means i.e. either by physical or chemical agents and these mutations are utilized in creating variability and new cultivars are developed. Mutation breeding has hence become a very useful technique in modern plant breeding (Shu et al., 2012).

The optimum quantity or dose of a particular mutagen is necessary for creating workable mutations and avoiding damage (Mba et al., 2010). In case of physical mutagenesis, the optimum dose depends on radio sensitivity of a crop. Radiosensitivity is the amount of radioactive radiations that can produce significant visible influences in the specified material (Oladosu et al., 2016). This is usually determined by exposing the subject material with different levels of radiation doses in different tests and the one that gives the best result is opted. Similarly, chemical mutagens are also tested for their efficiency and optimum doses. Different chemical mutagens like EMS (Ethyl methane sulfonate) and MMS (Methyl methane sulfonate) are used. They are applied to different sexually and asexually reproduced crop plants to produce mutations in them. The complete set of instructions, requirements and material is described in the protocol devised.

Types of mutations:

Mutations can be categorized in three broad classes i.e. Intragenic (mutations in single gene in DNA), Intergenic (chromosomal structural changes including deletions, duplications, translocations and inversions) and mutations causing change in chromosomal number. The mutations can also be divided in two types on the basis of their occurrence i.e. nuclear or plasmone.

1. Intragenic mutations:

Loss or gain of a single base pair or more causes deletion and insertion. Sometimes they cause shift of the entire frame of coding region so they are also referred as frame shift mutations. These mutations are of great importance because loss or gain of some base pairs can cause loss or gain of function of some genes. These mutations pose a significant impact on the genotype because the whole reading frame is altered due to change in one or more base pairs. The location of such frame shift mutation is also very important. If mutation occurs near 3' end it would lead to terminal part variation in the chain of polypeptide and an altered but functional protein may form. In some cases, the change of codon does not alter the amino acid because one amino acid can be encoded by more than one codon so there is no change in the protein sequence such mutations are called silent mutations. The mutations that change the protein sequence are called missense mutations. In second type of mutation the base pair is substituted with one another in the DNA sequence. Replacement of A by G or C by T can change the resulting codon and this can lead to change of amino acid and hence a modified protein would be formed and it will affect the functionality of that protein. The replacement of pyrimidines with pyrimidines is called transitions while substitution of pyrimidines with purines is called transversion. Transitions are more common than transversion (Mccallum et al., 2000 a and b).

2. Intergenic or structural mutations:

The structural changes or chromosomal mutations occur when the chromosomes break and then rearrange themselves. These are caused by ionizing radiations (Kumar and Yadav, 2010). Structural mutations are of four types.

a. Deletions:

The type of mutation in which some part of chromosome breaks and is lost is called deletion or deficiency. Ionizing radiations result in deletions and they are 90% of mutations caused by ionic radiations. These mutations are usually very deadly (Pathirana, 2011). Sometimes these mutations may be useful i.e. in impeding a pathway producing harmful plant product.

b. **Duplications**:

The type of mutation in which a part of chromosome is duplicated or repeated. Duplications lead to several changes in the genome for example in model plant like Arabidopsis have evolved and incorporated 3 major duplications in its genome (Bowers et al., 2003). Many other plants also exhibited this type of mutations in their genome (Barker et al., 2008).

c. Inversions

The type of mutation in which a part of chromosome breaks and then reattaches in reverse order. This type of mutation is 180-degree reversion of DNA segment. These mutations can cause severe disorders. Sunflower plant has exhibited 2 inversions in its genome (Heesacker, 2009).

d. Translocations

The type of mutation in which a part of chromosome breaks and attaches to some other chromosome. 5 major translocations in sunflower genome have been identified.

The chromosomal structural modifications and their evolutionary role has indicated that these mutations can be used to speed up the process of creating variation and selection of desired variation in developing cultivars. The practical evidence is provided by Sears when he transferred a segment of chromosome from Aegilops umbellulata and transferred it to bread wheat by bridge crossing using Triticum dicoccoides and ionizing radiations. The segment contained genes for brown rust resistance (Pathirana, 2011). The applications and uses demonstrate that the chromosomal mutations are very important in modern plant breeding.

3. Genome mutations/ Changes in chromosome number:

These are bigger changes which belong to whole chromosome addition or loss and hence change in chromosome number. These types of mutations are of prime importance in plant breeding (Pathirana, 2011). These types of mutations are of 3 basic types.

a. Allopolyploid:

When two different species are crossed the resulting individual possess genome of two different species or different genera. This type of change possess more than two copies of chromosomes as genome is inherited from two different species or genera. This type of ploidy is utilized in many plant species like, wheat, cotton and soybean (Pathirana, 2011). The evolutionary history of these crop plants clearly indicates the allopolyploid variations.

b. Auto polyploidy:

When chromosome number is multiplicated within the same species of organism and there are a greater number of copies of homologous chromosomes than 2. These mutations are exact multiples of the chromosome number. Different crop plants are autopolyploid for example cultivated potato tuber varies in ploidy level and shows 24,36,48,60 and 72 chromosomes count (Spooner et al., 2006). Similarly, banana has three sets of chromosomes and triploid in nature and is developed from allopolyploidy (Heslop-Harrison and Schwarzacher, 2007). In the same way, sugar beet is also studied at different levels of ploidy and it is reported that it gives best yield at triploid level (Kikindonov, 2009). This type of ploidy can be induced using agents that stop the spindle formation during the mitosis and hence the divided cell will have double number of chromosomes. The same technique is used in anther and ovule culture and breeding (Pathirana et al., 2011).

c. Aneuploidy:

This is the addition of one, two or three chromosomes in the cell and making abnormal number of chromosomes. This type of ploidy can produce 45 or 47 chromosomes in human cell instead of 46 chromosomes. These mutations pose lethal threats to the body.

Extranuclear chromosomes mutation: this type of mutation is present in the higher plants and this is change in the DNA of chloroplast and mitochondria. These mutations are also very useful in plant breeding. For example, in nicotiana plant antibiotic resistance has been introduced through plasmone mutations. These mutations are also involved in conferring many leaf and fruit traits to plants. The mutations that occur in mitochondria and chloroplast are not inherited in Mendelian patterns because they are transferred cytoplasmically (Pathirana, 2011).

Factors affecting the mutation induction:

The process is different for both sexually and asexually reproducing organisms. The success of mutation induction depends on following factors; state of the solution containing mutagen, the traits of subject experimental material, the environment of mutagenesis, quantity of mutagenic agents, solution

volume used for treatment, time period for treating the tissues, temperature of the process, pH of the process, soaking prior to process, catalytic agents used for the process and handling after the treatment of tissues with mutagens (Mba, 2013).

After completing the process there is need of taking care of mutated tissues. Factors which can influence the success rate are oxygen concentration, humidity, temperature and prevention from biological agents like bacteria and viruses. Following concepts should be clear before starting the process of mutation induction.

1. The traits controlled by single gene can be mutated easily as compared to the trait being controlled by many genes. Success rate is high in monogenic traits because it is possible to mutate a single gene.
2. The mode of reproduction should be clear because there are different methodologies for asexually and sexually reproduced organisms. In vitro or in vivo methods can be used for asexually reproduced organisms.
3. In sexually propagated plants seed is used as mode of distribution and the methods will depend either it is self or cross fertilized.
4. Selection of tissues for mutagenesis is an important step as different parts of plant i.e. seeds, leaves, stem or cuttings can be used.
5. The number of chromosomes sets in the target crop is also very important because ploidy level mutations can also be used.
6. Genetic architecture of the subject crop should be known and use of homozygous plants should be ensured.
7. In addition to all these the type of mutagens should be considered that what type of agent will be suitable. The laboratory conditions and equipment should be maintained as well.

The phenomena which causes abrupt genetic changes in living organisms is known as mutagenesis. Three types of mutagenesis were generally used physical (x-rays, gamma rays etc.), chemical (EMS and MMS) and site directed mutagenesis in DNA (Roychowdhury and Tah, 2013; Kharkwal and Shu, 2009). Genetic variation is very much needed in the plant breeding because this variation is exploited in choosing the desired traits. Mutant alleles also produce variations that is used as the source of genetic diversity in crop breeding. It is very important to locate the mutant individuals with targeted point mutations and then utilize them in breeding program. This has two phases of screening of mutants and confirmation of mutants (Forster and Shu, 2012). The mutant individuals are selected from the mutated population in first step of screening. Then the confirmation is observed through various tests (Mba, 2013).

Techniques in mutation breeding

1. Conventional mutation breeding method.

Stadler, (1928 and 1930) showed the use of radiations on crop plants as a tool for genetic variation by conducting experiments on maize, wheat and barley. He treated with X-Rays and Radium rays and proved that genetic mutations can be artificially induced. Physical mutagens generally include Gamma-rays, X-rays, alpha and beta particles, UV light and particle radiations, (Kodym & Afza, 2003). Gamma-rays, X-rays, thermal and wide neutrons (mainly obtained from P32 & S35) were widely used in successful induction genetic mutations (Gottschalk and Wolff, 2012). Alam et al. (2001) observed the heritable genetic changes with the treatment of gamma rays sourced from cobalt-60 and showed the use of radioisotopes as a potential tool for inducing genetic variation in plants.

After discovery of techniques & mutagenic properties of radiations, scientists started research on chemical sources. In World War I scientists observed similar mark of burns caused through X-ray and mustard gas. It was perceived by DR. J. M. Robson that mitotic disturbances were caused by the mustard gas. After experiments, decreased translocations in comparison with X-Rays and delayed effect was recorded (Auerbach, 1973). Now a days, diethyl sulfate (DES), ethyl-methane- sulfonate (EMS), N-Methyl-N-nitroso urethane (MNU) and ethylene imine (EI) are some potential chemical mutagens of which efficiency can be enhanced by combining them in appropriate manner. Seeds are the target for mutagenic agents for both chemical and physical mutagens in case of sexually propagated plants. Preference of physical agents were given for treatment to seeds because chemical agents affects the fertility of M1 plants (Gottschalk and Wolff
, 2012).

2. Space breeding.

Halstead and Dutcher (1987) reported chromosomal changes in dormant seeds and concluded those changes due to radiations and environmental affects not due to gravity. Dutcher et al. (1994) highlighted that gravitational force in space affected the development and growth at cellular and molecular level. Experimentation showed that cosmic radiations, supervaccum, microgravity and environmental conditions in space affects the plant growth, these also induced mutations in seeds with higher frequency and is a tool for genetic variation which can be exploited in various breeding programs (Liu and Zheng, 1997). Radiations in space and microgravity is a significant factor in the induction of changes in cell nuclei, DNA and other organelles. Spaceflight induces more mutagenic frequency and ratio in plants. Earth grown plants retrieved from space satellites have shown both genetic and physiological variations, (He et al., 2006). Luo et al. (2007) performed genetic analysis of rice plants retrieved from space satellite and summarized the effect of HZE (high atomic energy and power) particles on different parts of seeds. He found polymorphic locus in every plant but phenotypic mutation in only those plants where seed embryo was exposed. He identified Mg and Cr ions as a source of these changes in rice plants.

We can get the information of genes of organisms from sequence, but their phenotypes were hidden. Tilling is generally applicable where other reverse genetics approaches were impossible, does not have transgenic modifications and can also be used for agricultural applications rather in functional genomics (Henikoff et al., 2004). Main advantage of this technique is, its application to whichever specie no matter, the size of genome (specie ploidy level) providing point mutations of high frequency with random distribution throughout the genome and can play a role in discovering new alleles (Kurowska, et al., 2011). Gilchrist et al. (2013) reported that TILLING can be used to create genetic variation, a basis of plant breeding projects without any involvement of transgenic modifications and utilization of NGS technology is more successful for mutation identification in plant populations.

Liu et al. (2002) treated air dried wheat seeds with magnetic field-free space for 180 days. Seeds showed inhibited germination and growth, biological effects were also different from gamma-rays treatment. But this technique could stimulate the male gamete development when treated. Anther culture process obtains high quality of callus and high proportion of green plants.

3. Tilling.

Mccallum et al. (2000a) found a method which was fast, can be applied to any organism and provides a wide number of mutant alleles. He combined the efficient EMS (ethyl-methanesulfonate) for mutagenesis and a heteroduplex analysis for the detection of changed base pairs by DHPLC and termed the process TILLING (Targeting Induced Local Lesions in Genome). It is an efficient reverse genetics strategy which can be utilized for the production of phenotypic variants and genetic modification of crop plants, its steps are illustrated in figure 1. (Mccallum, et al., 2000b). Greene et al. (2003) analyzed 192 Arabidopsis Thaliana target genes with 1900 EMS induced mutations and reported that 99% of mutations were GC to AT transitions and were randomly distributed.

4. Eco-tilling.

Comai et al. (2004) adapted the TILLING technology for locating the polymorphisms in populations. He mixed the reference DNA with the DNA of an individual (1Kbp), target DNA region was amplified by fluorescent primers which were asymmetrically labeled by endonuclease CEL 1, heteroduplexes at the mismatched sites were nicked after the heating and annealing, Li-cor-gel analyzers were used for the visualization of cut strands, polymorphisms can be detected in single florescent channel termed as ECOTILLING.

Ecotilling is an efficient method for SNPs detection in natural population, but requirement of costly chemicals, equipment and special software limits its use. Alternative protocol is being adopted having simple gel system, standard data-gel analysis, unlabeled primers and DNA staining after digestion of single-stranded specific nucleases addressed those

limitations and performed successful identifications of SNPs in rice later on verified by sequencing (Kadaru et al., 2006). By ECO-Tilling we can also characterize the germplasm with less time and cost (Elias et al., 2009). In ECO-TILLING identification of natural genetic variation is done against the induction of mutations which is done in TILLING because in many species chemical mutagenesis cannot be persuaded so ECO-TIILING generally assists us to dig out natural alleles, its applications include association analysis, mapping, biodiversity and mutation profiling (Khan et al., 2018).

5. De-tilling.

Li et al. (2001) developed a new strategy to isolate and identify deletion mutants of target genes by using samples from deletion libraries generated by using fast neutron bombardment and screening it by PCR and specific primers. Amplification of deletion mutants and deletion alleles from pooled DNA samples can be identified by adjusting extension time of PCR allowing to deal a thousand mutant lines per sample (Li et al., 2002).

A reverse genetic strategy by inducing physical genomic deletions independent of size and exclusive recovery of knockout mutants termed as Deletion TILLING. Neutron based mutagenesis followed by sensitive PCR based screening which can be applied to every specie and can offer edge to crop improvement projects (Rogers et al., 2009). Steps are illustrated in Figure 2

De-TILLING can also be used as an isolating method for mutants accompanied with fast neutron mutagenesis creating knockdown mutants and is a low cost and simple method which can address the issues for targeting small genes (Wang et al., 2009). This technique can efficiently detect a single specific mutant from the pool of even 6000 plants (Shu, 2009).

6. Locus Targeted Mutations.

A simple method was developed in 1989 in which PCR based amplification of the plasmid by using specific primers was done which contained the desired changes and whole plasmid was amplified and generally suggested for the alteration of cloned genes (Hemsley et al., 1989). After this time megaprimer method was introduced in which three oligonucleotide-primers were used in two rounds of PCR reaction, product of first PCR cycle us used as the PCR-Primer (MegaPrimer) for second PCR cycle than the phage promotor accompanied by translational initiation signal attaches to appropriate primer resulting production of mutant protein without in-vivo manipulation (Sarkar and Sommer, 1990). An efficient megaprimer-PCR method is introduced later for site directed mutagenesis, primers were designed with different melting temperature “Tm” to prevent DNA purification by two rounds of PCR. In first reaction synthesis of megaprimer is done using mutagenic primer a low Tm primer having low temperature of annealing, in same tube second reaction is performed using high Tm primer and high annealing-temperature which actually prevents the priming of megaprimer with low temperature primer (Ke et al., 1997).

Targeted mutagenesis can be used to modify target genes in plants and other organisms. Zinc finger nucleases (ZFNs) based targeted mutagenesis found to be an efficient method which induce double stranded cleavage in the specific genomic sites than followed by repair mechanism which induces mutations, ZFN gene followed by Heat-shock promotor was introduced in Arabidopsis and after heat treatment 78% of deletions and 13% of insertions were reported (Lloyd et al., 2005). The DNA cleavage event is performed by two ZFNs custom-designed which heterodimerize on DNA forming a nuclease complex, these cleavage-competent heterodimers can address the off-target breaks that can affect the efficiency. Miller et al. (2007) reported an efficient ZFN methodology which uses two ZFN variants that breaks DNA only when heterodimer is formed modifying the endogenous locus efficiently making the ZFN architecture more specific. ZFNs can be efficiently used to cleave and induce mutations at specific locations (Jung et al., 2018) and can contribute in crop improvement programs.

By the fusion of Transcription-activator like effectors (TALEs) to the FokI endonuclease catylatic domain new class was reported which can cut DNA at targeted and specific sites (Christian et al., 2010). TALENs can be customized and these restriction enzymes can be used for induction of mutation (Hwang et al., 2014).

Targeted mutagenesis was done in Oryzuas latipes for DJ-1 gene homolog of homo sapiens DJ-1, TALENs induced number of deletions and insertions with high efficiency and transmissions to next generation was reported highly efficient. Site-specific mutagenesis was confirmed as no mutations were found at off-target sites (Ansai et al., 2013). TALENS is widely adapted technique for targeted mutagenesis in crop plants. TALEN was used to induce mutations at gl2 locus of Zea mays resulting mono and di-allelic mutations in genotype Hi-ll achieving frequency of about 10% in transgenic lines. It is an efficient tool for the induction of mutation and can be utilized for the improvement of traits and searching out gene functions, (Char et al., 2015). In rice double point-mutations were produced in gene OsALS using TALENs based on homologous recombination was heritable into next progeny and herbicide resistant lines were produced (Li et al., 2016).

CRISPR/Cas is one of the latest technologies which can be used as tool for locus targeted mutagenesis, first at targeted genomic sites by double-strand breaks were introduced which were than repaired by NHEJ (non-homologous end joining). It has the ability to induce heritable mutations and stable inheritance was demonstrated in Arabidopsis thaliana; by using two target sgRNA sequences for the guidance of Cas9 to the DNA strands off-target effects can be avoided (Schiml et al., 2014). Several generations possessing seven genes on 12 target sites was examined to check the heritability, specificity, efficiency and patterns. The proportion of plants having gene mutations induced by CRISPR/Cas was reported 71.2%, 58.3% and 79.4% at T1, T2 and T3 respectively. Homozygotes were found stable to next generation and no off-target mutations were reported and it was revealed that CRISPR/Cas is very useful method for the induction of novel and heritable changes at specific targeted genes (Feng et al., 2014). It is a general and applicable method which can allow us to introduce single nucleotide changes at targeted locus either they were essential genes or non-coding regions (Biot-Pelletier and Martin, 2016).

Managing off-target mutations and transgene integration are the important optimization targets; an efficient method was reported using CRISPR/cas9 RNPs (ribonucleoproteins) for the genome editing of bread wheat. Sequencing showed less chance of off-target mutations in RNP based methodology rather than CRISPR/Cas9-DNA. No off-target mutations were reported, and mutants were categorized as transgene free because no alien DNA was used (Liang et al., 2017). Process is illustrated in figure 3

Induction of variability and variety development through mutation breeding.

1. Wheat

Mutagenesis is a powerful tool which can be used to induce allelic variation and enhance genetic diversity. In bread wheat EMS was used to produce the population and by molecular characterization new allelic variants were reported (Sestili et al., 2010). Morphological and agronomic based study was done for the mutagenized spring wheat, TILLING method was applied to create the genetic variation and wide variation was reported in terms of selected morphological and agronomic characters (Rakszegi et al., 2010). In hexaploid wheat identification of desirable genomic changes on the basis of phenotype was problematic by reason of gene redundancy.

TILLING seemed as a promising approach in the case of wheat because of large mutation densities can be tolerated by polyploids. Treatment for three target genes was given by TILLING revealing 31 new alleles for those genes of which sequencing confirmed and mutation density by TILLING was 1/47kb (Chen et al., 2012). Dry seeds of Triticum durum was treated with gamma rays and high variation was reported in M1 generation with respect to days to flowering and maturity (Albokari, 2014). In Triticum aestivum L. narrow variation was present for amylose content hindering the improvement, the induction of variation for amylose content was done with the help of EMS-mutagen. Variation in resistant starch and amylose content was recorded and resultant population served as useful pre-breeding material which might be exploited in nutrition and quality improvement projects (Mishra et at., 2016).

2. Rice:

Chemical mutagens were widely used in the induction of genetic variation in crops specially for breeding projects. Effect of EMS on basmati rice was recorded and found efficient to induce variations, use of EMS can help us to create new germplasm (Muqaddasi and Arif, 2012). Successful screening of mutant lines was done, and tolerant mutants was identified showing less Na and K ions in their shoots as compared to sensitive mutants. Tolerant screened mutants were suggested potential varieties for the saline areas whereas genetic basis can be studied by cloning and mapping (Nakhoda et al., 2012). Two rice varieties were treated with six levels of gamma radiations as treatment out of which 200-Gy improved the number of grains per panicle of both varieties and enhanced the nutritional factors of only one variety (Masruroh et al., 2016). Use of CRISPR-Cfp1 was evaluated for two target genes OsBEL and OsPDS, method efficiently induced the heritable and specific mutations indicating the utilization of tool for mutagenesis in rice (Xu et al., 2017). For the development of haploid inducer in rice, ZmMATL (gene in maize responsible for intraspecific haploid-induction) orthologue OsMATL generated and by knockout mutation results showed reduced seed setting and haploid-induction rate of 2 to 6% indicating the functional conservation of MATL gene (Yao et al., 2018). Locus targeted mutation was performed in MIR396 gene-family, two regulators MIR396f and MIR396e for plant architecture and grain size were identified, these mutations can play a positive role in increasing grain yield and boosting leaf elongation (Miao et al., 2020).

3. Maize:

Chemical mutagenesis was found as promising approach to create variation for quantitative traits and stress tolerance. Genetic diversity of EMS based initial mutant lines and 3737 pioneer hybrid accompanied by RRMB (recurrent reciprocal mutation breeding) was evaluated by 18 SSR-Markers which revealed higher level genetic variation of mutant inbred lines than of classical inbreds with figures 0.61 for mutants and 0.608 for non-mutants. This indicates the use of mutagenesis as a valuable tool for the induction of genetic variation in maize breeding projects for stress tolerance and yield (Kostova et al., 2006). Mutation breeding was widely used for traits improvement. Maize inbred lines were treated with different doses of gamma rays and cytoplasmic connections for six inbred lines and phenomena of cytomixis was reported. This revealed the chromosome migration as an actual event and concluded that tool can possibly be utilized for polyploid, aneuploid production and enhances the diversity (Rai et al., 2010). Specific genes namely ZmPDS, ZmIPK, ZmIPK1A, and ZmMRP4 were targeted in Zea mays L. using TALENs technology. The targeting efficiency reported in protoplast was about 23.1% and 13.3%-39.1% were somatic mutations. ZmIPK gene in protoplast is targeted by CRISPR/Cas using two gRNAs and revealed the efficiency for induced mutation in protoplast i.e. 13.1% almost similar to TALENs which was 9.1% (Liang et al., 2014). Treatment of sodium azide was given to seeds and variation was reported for agronomical and morphological traits; correlation analysis showed strong positive correlation for number of cobs, husk cover and ear aspect with mutation tolerance while grain weight, shoot biomass, stem height and root biomass were nearly related with mutation tolerance in principle component analysis. Author suggested to select the sodium azide tolerant genotypes for breeding projects (Olawuyi & Okoli, 2017). Phenotypic screening was done for the EMS mutagenized population, mapping-by-sequencing was done rapidly for the fascinating eat mutant which identified a deletion in promotor ZmCLE7. EMS mutagenesis followed by mapping-by-sequencing can be used to discover novel genes and perusing forward genetics in germplasms of diversified genetic backgrounds (Tran et al., 2020).

4. Pulses:

Effect of EMS on Vigna radiata and Vigna mungo showed that EMS-mutagenesis is an efficient tool for the induction of mutations. Selection based on genetic variation in yield parameters and yield contributing traits was also performed and linear correlation was reported for yield, seed protein-content and NRA (Kozgar et al., 2011). Pulses holds principle status in global agriculture due to phenomena of nitrogen fixation in atmosphere and high content of protein. Artificial induction of mutations was one of the best ways to widen the genetic variation which can be further utilized for the development of high yielding varieties. Mutation assists the researcher to restore that

variation which was lost in evolutionary processes (Auti, 2012). EMS-mutagen treatment on cowpeas showed that chromosomal and morphological variation were mutagen sensitive in the somatic-cells which increased when EMS concentration was increased (Gnanamurthy and Dhanavel, 2014). Bohra et al. (2014) stated that multipurpose status of pulses provides food security in impoverished and food deficient regions. Low progress in minor-pulse crops was reported though they can be grown in rain fed areas with least inputs.

Narroe genetic diversity in chickpea, which is mainly because of self-pollination nature is main problem which hinders the development. By mutation breeding we can induce variation within short time span which can provide us reasonable grounds to combat food security and other emerging problems (Raina et al., 2019). Lentil has been grown through ages and is an outstanding food source, which provides good nutrition. Selection and domestication over ages resulted in low genetic variation limiting the scope of lentil breeding. Mutagenesis can be utilized to induce the variation and can improve the lentil genetic resources which can further lead to the production of improved varieties (Laskar et al., 2019).

5. Ornamental Plants:

Floriculture industry holds special status especially in developing countries. Flowers with good colors, decent shape and long shelf-life were demanded by customers and to meet the need genetics resources of ornamental crops could be improved by utilizing mutagenesis and biotechnological tools together (Mohan Jain, 2006). In-vitro grown dendranthema grandiflora was treated with gamma rays and change in flower color from white to yellow was reported. Regeneration of mutants was also stable showing that potential mutants can be developed in short time span (Kaul et al., 2011).

Mutation breeding is very successful method in ornamental plants due to easy phenotypic screening. Ornamental plants are mostly heterozygous and the most promising one is vegetative propagation of varieties. Chimera formation in propagated plants was reported as a constraint in mutation breeding (Datta, 2012). In-vitro mutagenesis of tea rose hybrid is performed with gamma rays, examination of morphological traits showed four mutants having altered and new flower color as compared to original flowers followed by large-scale multiplication by micro-propagation to check the stability (Bala and Singh, 2013). Kainthura and Srivastava (2015) treated tuberose varieties with EMS, X-rays and Gamma-rays reporting significant effects on parameters induced by low doses of mutagens and six mutants were gained on the basis of variation. Comparative study of best effecting mutagen was performed; gladiolus corms were treated with different doses of gamma-rays and DMS. DMS generally targeted vegetative characters followed by less survival-rate as compared to gamma rays and plants were more responsive to it (Patel et al., 2018)

6. Other Crops:

Kumar et al. (2012) reported variation in mutants of Brassica napus L. on the basis of characters which contained yield, number of branches, 1000 grain-weight and number of siliquae. Mutants with enhanced level of oil content were also observed. Random mutagenesis in polyploid species were ineffective because of gene redundancy. ALC homologs were targeted by CRISPR/Cas9 to produce shattering resistance. In T1 four mutant alleles of ALC were reported (Braatz et al., 2017).

In-vitro gamma-ray mutagenesis followed by salt-tolerance screening in sugarcane was performed; some of the mutant clones showed enhances sugar yield, brix % and millable canes (Nikam et al., 2015). Gamma radiation treatment was given to buds of sugarcane and significant differences were reported for traits. Lower doses of gamma rays were reported more efficient for the induction of variation (Kaur et al., 2016). Treatment of EMS was given to sugarcane callus followed by PEG treatment to induce drought stress. Total 18 lines were selected and planted in greenhouse for trial in which seven lines survived again which proves that this technology is practically applicable for crop improvement (Masoabi et al., 2018).

Gamma-ray treatment was given to tomato seeds to induce heat tolerance and identify high-yielding varieties, screening was done till M6 generations on the basis of characteristics and 20 lines were successfully identified which exhibited high yield and

stress tolerance (Saraye et al., 2018). EMS mutagenesis was applied in Gossypium herbaceum and tilling population was derived, morphological data revealed more variable CV, mean performance and range of dispersion in mutants. Phenotyping exhibited that 31.6% plants contained little-variation and 2.2% have visible changes in morphology (Umesh and Hemant, 2018). EMS and Gamma-ray mutagenesis were used to induce lodging resistance and photosynthesis efficiency in kodomillet; significant variation with respect to morphological-traits was reported and later on expression profiling revealed mutation induced different level of expressions in same genes belonging from different lines (Jency et al., 2020).

Conclusion:

Food security, climate change, biotic & abiotic stresses, crops having narrow genetic base and food crops unable to withstand against challenges are the major problems and are the ultimate objectives for breeders to deliver in crop improvement schemes. Mutation breeding provides us firm grounds in terms of widening the genetic variation. Genetic variation might lead the breeders to produce and identify the desired potential combinations in very short period. Number of affective methodologies present for mutagenesis assist us to run projects in under-developed and resource-deficient laboratories. It is a good tool to exploit variation in those crops where natural breeding projects cannot be performed or of least interest due to low success rate. It contains lots of potential which can be utilized in crop-improvement programs.

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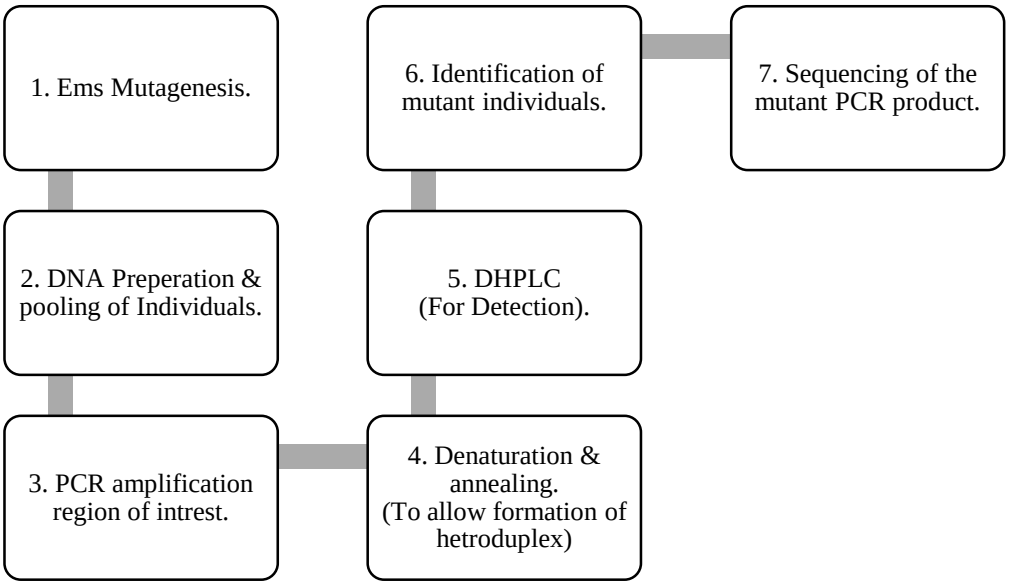

Figure 1. **Main steps of Targeting Induced Local Lesions in Genome (Mccallum *et al*., 2000b).**

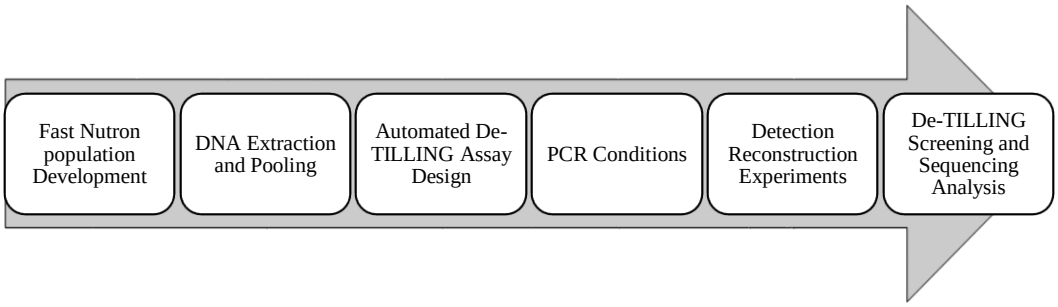

Figure 2. Steps involved in DE-TILLING (Rogers *et al*., 2009)

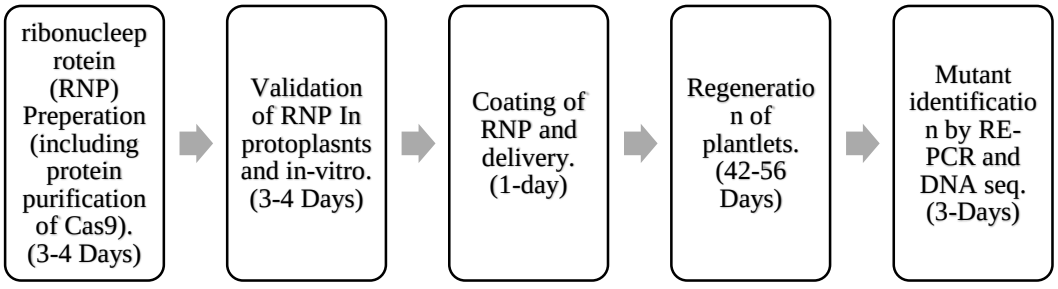

Figure 3. Structural outline of CRISPER/Cas9-RNP for the induction of targeted genetic mutations in wheat (Liang *et al*., 2017).

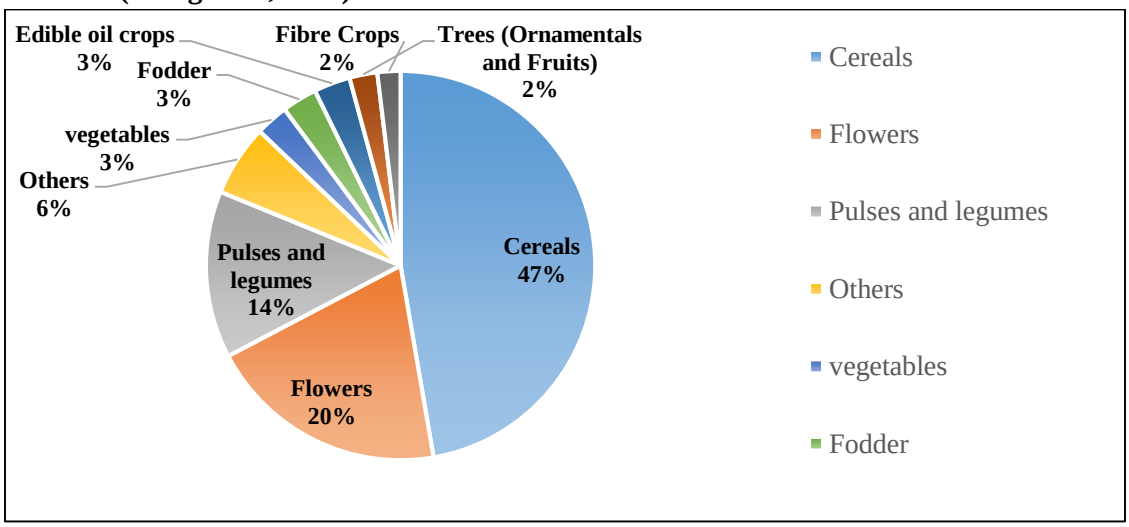

Figure 4. Varieties produced by mutation, officially released and reported on FAO/IAEA joint mutant-varieties database (Mutant Variety Database, 2020) (https://mvd.iaea.org/).