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Review Article

Review on Mutation Breeding in Legumes and Nodulation Mutants of Different Legumes

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A B S T R A C T

In the present situation of increasing population and depleting cultivatable land and water resources and unpredictable rainfalls food insecurity is the major challenge faced globally for the human existence. This food insecurity was further enhanced by the major climate change that plays an important role in reducing the food production. Developing climate smart varieties may help us in increased food productivity by their broader adaptability to range of varied ecosystem. Genetic variability in existing plant cultivars and germplasm collection is the result of selection process during evolution and plant breeding. Mutation has been considered to play a prominent role in evolution. Mutagenesis offers the plant breeder a chance to equip for unconventional objectives, particularly those that were at a selection disadvantage in the past. There are wide range of mutagens available for induction of mutation in crop plants and there by useful for crop improvement. Legume mutation breeding has already resulted in production of cultivars that has Better grain quality, high yield, and also resistant to pathogen. Several mutations that affect the process of nitrogen fixation related characteristics have also been recorded. In nature mutation is the major cause for the variation and without that plant breeding will be very difficult. Increasing productivity and quality is the main focus by developing well adapted plant cultivars by altering some major characters through mutation breeding. This reviews the progresses in the improvement of legume crops through mutation breeding. Types of mutations and mutagens like the physical and chemical mutagens their effects and procedure for mutation breeding handling of mutated populations and Nodulation mutants in the legumes were discussed. This review highlights the role of mutation breeding in world and legume crop improvement and thus helping us in overcoming the problems of food security due to rising population.

Keywords

Mutation, Mutation breeding, Mutagens, Legumes, Food security

Introduction

Pulses which are also known as grain legumes are valued for their protein richness and are important for the human diet. Grain legumes became important components in sustainable agriculture due to their potential to survive well under different climatic conditions, soil fertility restoration capacity and soil ameliorative features. In India several pulse crops are cultivated over an area covering nearly 22-24 million hectares. Nearly 75% share of production comes from the states of Uttar Pradesh, Madhya Pradesh, Maharashtra, Andhra Pradesh and Rajasthan. Here are some important pulse crops with their respective share field pea (5%), lentil

(7%), urdbean (10%), mungbean (12%), pigeonpea (16%), chickpea (38%). India is the largest producer of pulses in the world contributing nearly 13-15 million tones to the worldwide production.

Plant breeders thought the country are following various techniques for crop improvement like hybridization, selection and mutation in pulse crops for creating variation and designing of genotypes with high yield potential. Several varieties were released through mutation because of this out of all breeding methods mutation is considered as potent tool for creating variation. This review paper mainly focused on mutation breeding basis and its applications covering the previous work done in mutation and achievements in the form of released varieties in pulse crops.

Mutation

Mutation is a sudden heritable change in the genotype of an organism and plays a role in the evolution. In 1901 Hugo de Vries first used the term 'mutation' to illustrate the phenotypic changes that were heritable. The mutagenic effects of X-rays were studied in *Drosophila* by Muller (Muller1927). In USA the first legume in the world a variety of groundnut that is N.C. 4-X as a mutant variety by X-rays induction was released in the year 1959 (Gregory 1955).

Mutation can occur either spontaneously or by artificial induction. It is now used only to cover the changes that alter the chemical structure of gene. They are commonly known as gene or point mutations. Induced mutants are used in the breeding methods to develop superior varieties known as mutation breeding and is used widely for the development of new crop cultivars and to change the plant traits. A large number of mutants are induced by the use of radiation and chemicals for the production of gene expression, genetic maps and gene regulation (Ahloowalia and Maluszynski, 2001). The mutations are induced by several agents called as mutagens. Mutator gene promotes mutation in other genes. Chromosomal mutations are the change in the structure of the chromosomes like deletion, translocation, duplication and inversion. Based on the magnitude of phenotypic effect the mutations are divided into Macro mutations and micro mutations (Gaul, 1964).

Forms of mutations

They are classified into two types. They are

Induced mutations

Mutation is induced artificially through mutation breeding by the treatment of mutagen directly to the plant or plant parts like seeds, pollen, ovules or stem cuttings.

Spontaneous mutations

It is an important source of genetic variation naturally. It occurs without any exogenous application or treatment of mutagen. Due to the error of replication these mutations occur. They are recessive, very rare and not sufficient to meet the requirements of crop improvement.

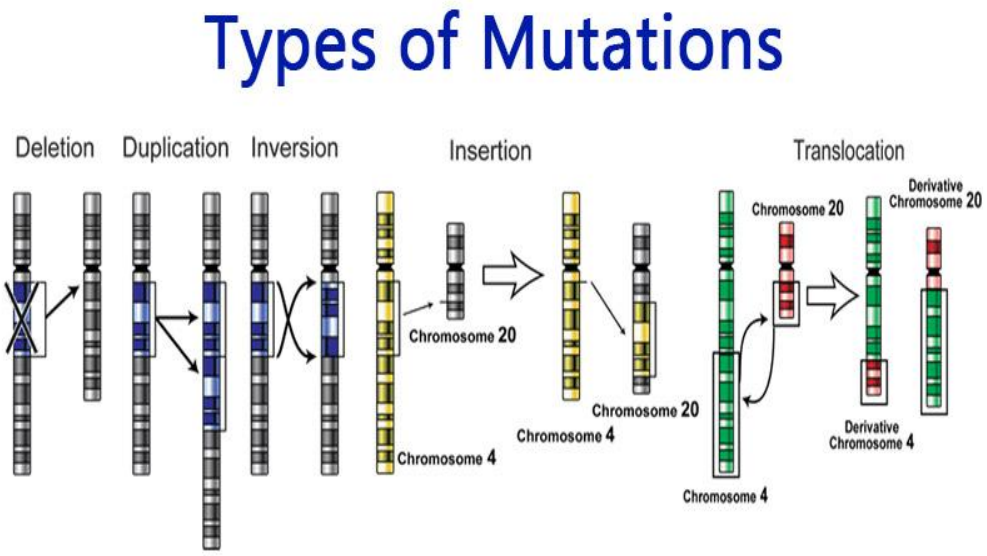
Mutations are mainly divided into two types:-

A) Intragenic or point mutations:-These occur within the gene of DNA sequence.
B) Intergenic (or) structural mutations within chromosomes these can be

1. Inversions
2. Translocations
3. Duplications
4. Deletions.

Mutations can be a result of the following

a) Deletion
b) Duplication
c) Inversion
d) Insertion
e) Translocation



Types of mutations

Two types of mutations they are

- Macro mutations
- Micro mutations

Macro mutations

- It is measured at individual level with a number of changes in the characters that can be detected without any instruments. They are oligogenic and can be selected easily in the M2 generation.
- Morphological and lethal mutations are included in these macro mutations.

Micro mutations

- It is measured at population level and changes occur in the quantitative traits. More attention is given to these mutations by the breeder due to the genetic variability in the quantitative traits.

Based on method of their detection

Morphological mutations - It can be change in size, color, shape, form etc.
Example: In Pea Dwarfism.

Lethal mutations - It results in death of the individual due to the change in genotype. They are easy to score.

Example: Albino ones are obtained from chlorophyll deficiency is lethal.

Biochemical mutations - This can be prevented by supplying nutrient to which the mutant is recognized to be deficient.

Mutation breeding

When the induced mutations are used to create new variations in crops with desirable characters then that approach is called mutation breeding.

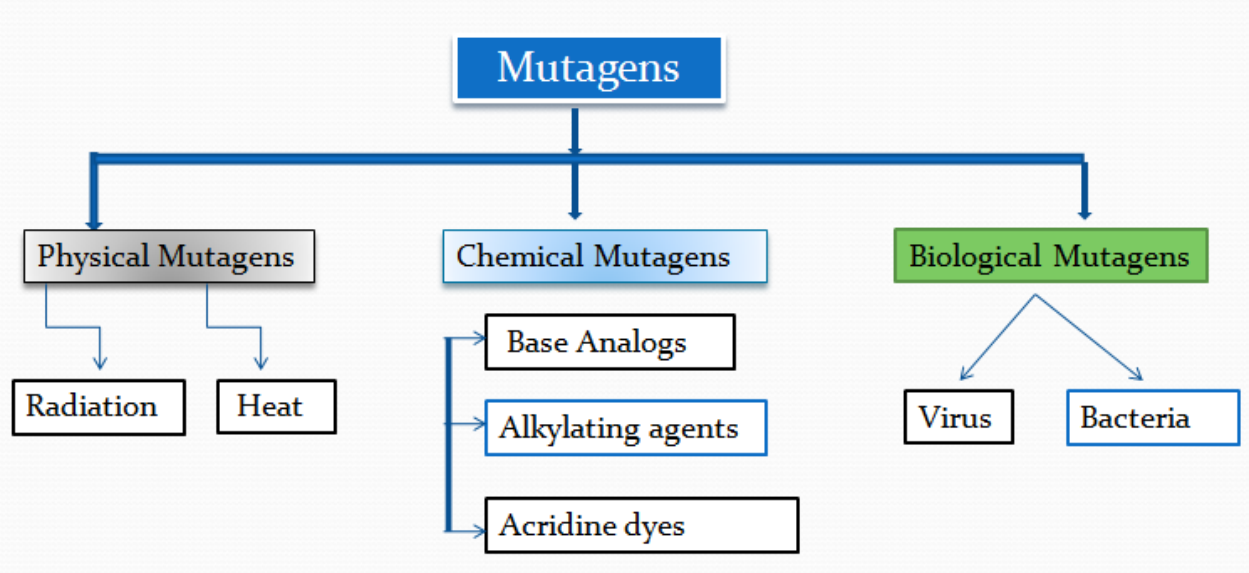
Importance of mutation breeding

The mutation breeding plays an important role in developing high yielding varieties and ideal plant type (Sarwar *et al.*, 2006).

Mutagens

Chemical or physical agents which are used to increase the frequency of mutations are known as mutagens.

Mutagen types



Physical mutagens

Radiations are of two types based on their energy levels:

Non-ionizing radiations: Lower energy level radiations are able to cause excitations at the nitrogen base level in the genetic material
Example: Ultra violet light etc.

Ionizing radiations: Higher energy level radiations are able to cause both ionization and excitations at the level of nitrogen bases.

Example: alpha particles, Fast and thermal neutrons, beta particles, X-rays, gamma rays, etc. (Table 1).

Chemical mutagens

The chemicals that aid in increasing the frequency of the mutations are called as chemical mutagens. Based on the nature of action the chemical mutagens are classified into three categories.

Alkylating agents

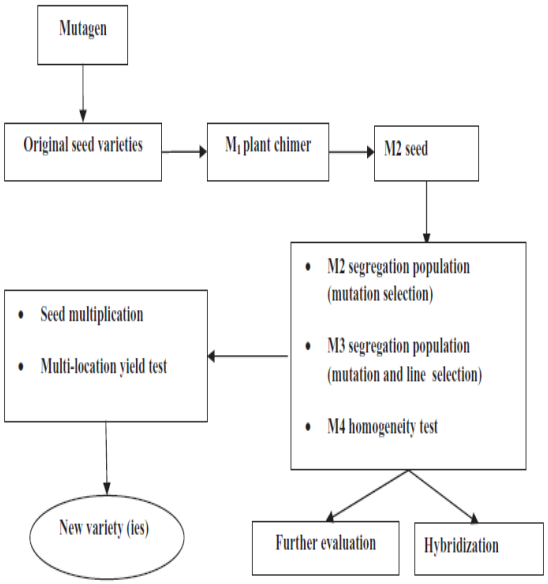
The addition of an alkyl group to the nitrogen bases results in mutation.

Example: Ethylene Imines (EI), Methyl Methane Sulphonate (MMS) and Ethyl Methane Sulphonate (EMS) etc.

Base analogs: These are the chemical analogous to nitrogen bases. They can cause wrong base pairing that results in mutations when incorporated into the DNA at the time of replication.
Example: The common base analogues used as mutagens are 5-bromo uracil and 2-amino purine.

Acridine dyes: Chemicals that get inserted in between two bases of the DNA at the time of replication thus results in either the addition of a base pair or deletion of single or a few base pairs.
Examples: Acriflavin and Proflavin.

Procedure of mutation breeding



1. Selection of the material
2. Choice of the mutagen
3. Mutagen Treatment
4. Handling of the mutated populations in the case of seed propagated species
5. Handling of mutated populations in the case of clonally propagated species

Selection of the material

First step in mutation breeding is to decide the nature of variations that are to be induced and to select the suitable material for mutagenic treatment. Based on the method of propagation that is seeds or other propagules are to be selected for the treatment. Callus or other *in vitro* material is selected for *in vitro* mutagenesis.

Choice of mutagen

The Selection of mutagen is done based on the nature of action of the mutagen and the mutation type to be induced. For vegetative propagules and pollen Radiations are ideal and for seed treatment chemicals are used to induce mutation.

Treatment of mutagen

In Chemical treatment, the pretreatment of materials is done by presoaking in water or other chemical solutions to enhance the effect of mutagens. These materials are later transferred into the mutagenic solutions. The Concentration of mutagen near to LD_{50} is regarded as optimum. The source of the mutagen is kept at a distance and the treatment is controlled in physical mutagenic treatment. The Gamma gardens are the experimentally protected areas where the treatment is carried out by Gamma rays. The duration of treatment is decided based on the information available. Lethal dose (LD_{50}) of mutagen causes mortality in 50 percent of the treated materials (All the mutagens are toxic and results in considerable death and deformities to the biological systems).

Handling of mutated population in case of species propagated by seeds

M1 population is produced from the germinated seeds. Most of the mutations can be selected only in later generations as they are recessive. However, in M1 population we can select both dominant and pseudo-dominant mutations. Seeds obtained by selfing the M1 plants are harvested separately. The collected seeds collected from M1 generation are then raised for M2 generation. At this level oligogenic mutations are also selected. These seeds are grown alone and beneficial mutants are isolated after required trials are done. M2 plants that are

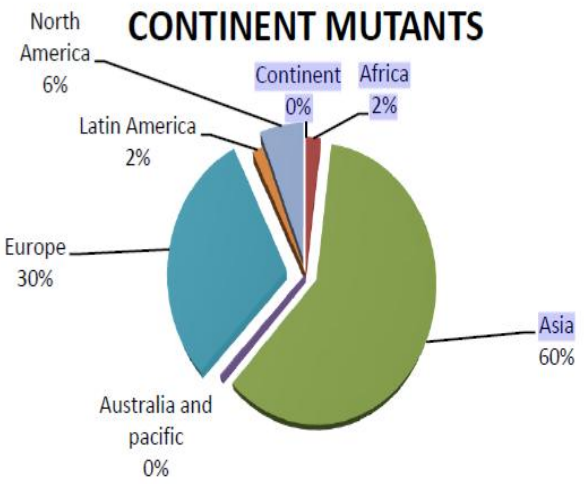
Superior are selected and the M3 seeds are then collected. The M3 progenies are raised from the M3 seeds and their breeding behavior is studied. Yield trails are conducted on the seeds of true breeding progenies are bulked together. Preliminary yield trials are conducted in the M4 generation. Co-ordinated yield trials are carried out from M5 generations onwards. Most of the promising lines are selected and released by the M8 or M9 generations. Based on the screening tests at M3 and M4 levels the plants that are inferior are rejected (polygenic traits). The remaining seeds are then bulked, used for yield trials and are released as new varieties finally.

The Handling of mutated populations in case of clonally propagated species

In the vegetatively propagated species the mutations are expressed as chimeras. They are combinations of genetically different tissues. The plants raised from the treated propagules are called as VM1 generation in case of vegetatively propagated crops. The plants having chimeras are selected and propagated for the production of VM2 generation. The solid mutants are recognized and selected in the VM2 generation. In VM3 generation, the identified mutations of VM2 generation are confirmed. Preliminary yield trials are carried out from VM4 and the coordinated trials from VM5 generation. By VM9 generation the best line will be released as a new variety.

Number of mutants developed in several countries. Data source: FAO mutant variety database February 2018

China	810	Pakistan	57	Turkey	10
Japan 479	479	Canada	40	Egypt	9
India	335	France	39	Australia	9
Netherlands	176	Indonesia	29	Myanmar	8
United States	139	Iran	24	Malaysia	7
Germany	171	Austria	17	Mexico	5
Bangladesh	70	Brazil	13	Sri Lanka	4



Percentage of officially released mutants across the Continents

Source: - FAO mutant variety database, February 2018

Mutation in pulses

Pulses are poor man's meat. They are the most important source of vegetable protein in India. Grain legumes are known for their higher protein content in their seeds. They are grown in the soils with poor in Nitrogen content that fix atmospheric nitrogen to ammonia used by plants. Biological nitrogen fixation (BNF) is an important component of nitrogen cycles in Pulses. The crop yield increases by increasing the nitrogen fixation by legumes (Danso, 1995; Fried, 1995; Herridge *et al.*, 1995b; Boddey *et al.*, 1997). Nearly 408 mutant varieties of legume crops that belong to 24 different crop species have been released using mutation approaches for cultivation worldwide (Kharkwal *et al.*, 2005).

There is significant role for mutation breeding in pulses as the variability in germplasm of pulses is less and are used to create variability. In the past to study the mutagenic effects of grain legumes both physical and chemical mutagens are used as a

result of varietal development. It has contributed more than 34 varieties for commercial cultivation of different pulse crops. At Global level nearly 97 varieties are developed (Gopala Krishna and Reddy, 2009). Most of the varieties are developed through the use of gamma rays (Table 1).

The use of induced mutants is widely used these days in several breeding techniques to transfer the required trait. Some of the important achievements in the improvement of pulses through the mutations are given in Table 2.

Nodulation mutants in legumes

Freiberg *et al.,* (1997) reported that the sequenced536kb plasmid NGR234 taken from a strain nodulated over 100 legume genera.

First isolation of mutants that can nodulate in the presence of higher nitrate concentration was done by Caroll *et al.,* (1985) after the treatment of soybean seeds with ethyl methanesulfonate (EMS).

Nodulation mutants are mainly classified into four classes (Sagan *et al.,* 1994):

- Nod- no nodules
- nod+/- few or no nodules
- fix- ineffective nodulation
- nod++ -supernodulation or hypernodulation
- nts(nitrate tolerant symbiosis) nitrate tolerant nodulation.

In addition, other mutants that are deficient in nitrate reductase and resistant to mycorrhiza are

- Ethylene resistance (insensitivity) (etr)
- Mycorrhiza resistant (myc)
- Nitrate reductase deficient (nar)

Table.1

S.no	Mutagen	Characteristics	Hazard
1	X-Rays	Electromagnetic radiation; penetrates tissues from a few millimeters to many centimeters	Dangerous, penetrating
2	Gamma Rays	Electromagnetic radiation produced by radioisotopes and nuclear reactors, very penetrating into tissues, sources are 60Co (Cobalt-60)and 137Cs (Caesium-137)	Dangerous, and highly penetrating
3	Neutrons	There are different types (fast, slow, thermal), produced in nuclear reactors, uncharged particles, penetrate tissues to many centimeters and source is 235U	Very hazardous
4	Protons	Produced in nuclear reactors and accelerators, derived from hydrogen nucleus and penetrate tissues up to several centimeters	Very dangerous
5	Alpha particles	Derived from radioisotopes, a helium nucleus capable of heavy ionization and very shallowly penetrating	Very dangerous
6	Beta particles	Produced in particle accelerators or from radioisotopes	May be dangerous

Table.2 Mutant varieties developed through different mutagens in the world

Crop	Gamma rays	X-rays	Ems	Others	Total
Chickpea	12	0	0	2	14
Pigeonpea	1	1	0	4	6
Mungbean	18	0	0	14	32
Urdbean	1	0	0	7	8
Pea	6	3	0	25	34
Lentil	1	1	0	1	3
Total	39	5	0	53	97

Symbiosis mutants in pea

S.No	Parent / Mutagen	Main characteristics	References
1	*Sym 1*	Temperature sensitive nodulation and resistant to strains below 20^0 C	Lie, 1971, 1984
2	*sym-6*	Ineffective nodulation, monogenic recessive	Caetano-Anolles and Gresshoff, 1991
3	*sym-8*	Chromosome 6 is essential for the induction of early nodulating genes like PsENOD5 and PsENOD12A Non-nodulating.	Albrecht *et al.*, 1998
4	*sym-13*	Ineffective nodulation, monogenic recessive, Chromosome 2	Kneen *et al.*, 1990
5	*Sym 2*	Nodulation resistance to a large number; of European strains, dominance dependent upon Rhizobial strain, Chromosome 1. Arrest in infection thread, if *Rhizobium* strain does not produce Nod factors	Holl, 1975 Geurts *et al.*, 1997 Lie, 1984 Kneen and LaRue, 1984

Nodulation mutants of soyabean

S.No	Parent / Mutagen	Main characteristics	References
1	SS-2	Super-nodulation at early stages than *nts*1007 and *nts*1116, more nodule mass, higher C2H2 activity	Hong Suk *et al.*, 1997; Hong Suk *et al.*, 1998; Hong Suk and Suk Ha, 1998
2	*Rj*2	It is Monogenic dominant	Caldwell, 1966; Caldwell *et al.*, 1966; Vest, 1970; Vest &Caldwell, 1972 and Devine, 1984
3	Non Nodulating5 (*rj*5 *and rj*6)	No nodule formation when inoculated with *Bradyrhizobium japonicum*, non allelicto *rj1*, allelic to *nod*139, recessive a non nodulating mutant of Bragg. No nodule formation in the field.	Mathews *et al.*, 1989; Pracht *et al.*, 1993

Nodulation mutants of other legumes

S.No	**Parent/ Mutagen**	**Main Characteristics**	**References**
1	Rabat Non Nodulating (*rn*8)	Nod-, mr	Singh &Rupela, 1998
2	Annigeri Non Nodulating	Nod-, mr	Singh &Rupela, 1998
3	PM 233 (*rn*1*)*	Nod-, mr	Davis *et al.*, 1985, 1986;
4	PI 109839	Trigenic	Dutta and Reddy, 1988
5	I 40(*Sym-2*)	Nod-, monogenic dominant	Esser-Monning *et al.*, 1995
6	Swan Valley 145	NTSN, mr	Park and Buttery, 1989b, 1994

Achievements of legume mutation breeding

Many goals have been reached by mutation breeding of grain legumes. Many mutant cultivars of *Phaseolus vulgaris* have been releasedas well as soybean-43, groundnut-33, pea-27, lupin-18, mungbean-9, cow-pea-9, faba bean-8, chickpea-7, pigeon pea-5, etc., these possess many different improved characters such as disease and insect pest resistance, earlier or late

flowering, high yield, higher protein content or less toxic compounds. (FAO/IAEA, 1988; Jaranowski and Micke, 1985; Micke *et al.*, 1985; Micke and Swiecicki, 1988).

In conclusion, according to the database on released mutant cultivars since 1950shows specific improvements in the activity on radiation induced mutations in over 70 countries. By induced mutations 3222 mutant varieties were registered in more than 232 different crop and plant species. Among these largest number is in China released - 810, followed by Japan - 481 and India - 330.The availability of accessible genetic variation is highly essential to initiate crop improvement programme. Induced mutagenesis is one of the most powerful breeding techniques for creating new genetic variation and accelerating the process of character selection. The main aim is to identify the legume mutants that are ideal to grow under different climatic changes and resistant to both abiotic and biotic stresses along with the higher yield to avoid low productivity. With advancements of modern biotechnology and genetic markers the unbound possibilities of mutation breeding are increasing in future, It is highly recommended to incorporate molecular advancement into the mutation breeding programme for improving the selection accuracy and target trait specificity.

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