Novel Mutation Breeding Techniques And Their Application In Vegetable Crop Improvement.

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Abstract - Mutation breeding is a crucial method for crop improvement that leverages induced genetic diversity to enhance various agronomic traits. Originating in the early 20th century, this technique utilizes physical mutagens like gamma rays and chemical agents such as Ethyl Methane Sulfonate (EMS) to introduce genetic variations. Recent advancements include genome editing, insertional mutagenesis, and antisense mutagenesis, offering enhanced precision in mutation control. Research demonstrates the effectiveness of mutation breeding in improving crops: for example, EMS-induced mutations have increased anthocyanin content in eggplant, while laser irradiation has enhanced growth traits in brinjal. Notable successes include the development of high-yielding potato 'Nahita' and disease-resistant cucumber 'Xianghuanggua 5.' Despite its advantages, such as cost-efficiency and rapid trait development, mutation breeding faces challenges including low mutation frequency and potential side effects. Continuous advancements in screening methods and integration with genomic tools are advancing the efficacy of mutation breeding. This approach plays a significant role in addressing genetic limitations, contributing to food security, and promoting agricultural sustainability by developing resilient and high-performing crop varieties.

Key - Vegetables, Mutation, Mutagenesis

I. Introduction

Vegetable breeding can be broadly classified into three subtypes: mutation breeding, recombination breeding, and transgenic breeding. Mutation breeding, in particular, is characterized by the generation of new mutated alleles. The concept of "mutation" was first introduced by Hugo de Vries in 1900 to describe inheritable phenotypic changes. The practice of using induced mutations for crop improvement, known as mutation breeding, gained significant traction during the 1950s, especially in the United States, Europe, Japan, and China. In India, a notable program on mutagenesis in crop plants was initiated by M.S. Swaminathan and his team at the Indian Agricultural Research Institute in New Delhi.

The process of mutation breeding involves key steps such as analyzing the sensitivity of different genotypes and plant tissues to various mutagenic agents, often measured using lethal doses (LD). Mutations can induce both qualitative and quantitative variations in a relatively short period by altering alleles at known loci and at previously unknown loci, in addition to modifying linkage groups. Induced mutagenesis has been employed to obtain direct mutants or to use these mutants in hybridization efforts, thereby overcoming yield plateaus and generating desirable horticultural traits. The study of induced variability, particularly for chlorophyll and viable morphological mutations in the M2 generation, has proven to be a reliable method for utilizing beneficial mutations in crop improvement.

The significance of mutations in plant breeding became more apparent following the discovery of the mutational effects of X-rays on Drosophila flies by H.J. Muller in 1927. The first crop species to undergo mutation was barley, with L.J. Stadler using X-rays to induce mutations. Early work with ionizing radiation and chemical mutagens in vegetable crops primarily focused on determining the optimal dosage and exposure of various agents to induce a high percentage of mutations without causing malignancy.

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Types of Mutation

Mutations in plants can be classified into several types: gene mutations, chromosomal mutations, cytoplasmic mutations, and bud mutations.

Based on Occurrence:

- **Spontaneous Mutations:** These occur naturally within populations at a relatively low frequency, generally around 10^{-6}.

- **Induced Mutations:** These are deliberately induced through the application of physical or chemical agents, known as mutagens.

Characteristics of Mutations:

- Most mutations are recessive, with dominant mutations being rare, examples of which include epiloia in humans and Notch wing in Drosophila.
- Mutations occur randomly, both in terms of timing and the specific gene they affect.
- When mutations occur simultaneously at both loci, mutants can be detected in the M1 generation. If a mutation is confined to one locus (shifting from dominant to recessive), its effects are usually observed only in the M2 generation.

Mutagens

Mutagens are agents that cause changes in DNA, resulting in mutations. These are generally classified into two categories: physical mutagens and chemical mutagens. Gamma radiation and Ethyl Methane Sulfonate (EMS) are the most commonly utilized mutagens in crop improvement programs.

Physical Mutagens:

Ionizing radiation, which disrupts the DNA's double helix structure, is a widely used physical mutagen. Common forms include X-rays, gamma rays, and neutrons. Of these, gamma rays are the most frequently used due to their high penetration ability, high mutagenic frequency, ease of use, and minimal disposal challenges, making ionizing radiation particularly advantageous for inducing mutations.

Chemical Mutagens

In response to the need for avoiding aberrations and harmful effects associated with ionizing radiation, researchers discovered a variety of chemical mutagens capable of inducing permanent changes in DNA bases. Key chemical mutagens include Ethyl Methane Sulfonate (EMS), methyl methanesulfonate (MMS), sodium azide, and mustard gas. Unlike irradiation, chemically induced mutations are widely favored for their ease of use, as they do not require specialized equipment. These mutagens effectively increase mutation frequency by creating point mutations, single base-pair changes, or single-nucleotide polymorphisms.

Chemical mutagenic treatments can be applied to seeds as well as in vitro cultured tissues of various plant types, such as corms, rhizomes, bulbs, tubers, and vegetative propagules. Ethyl Methane Sulfonate, in particular, modifies DNA by adding an ethyl group to guanine or cytosine, causing the DNA replication machinery to misread these modified bases as adenine or cytosine, respectively, leading to mutations.

Novel mutation breeding techniques

1. Induced mutagenesis Induced mutagenesis involves deliberately altering an organism's genetic material using external agents called mutagens, which can be either physical (e.g., radiation) or chemical (e.g., alkylating agents). This process, widely used in research, agriculture, and biotechnology, creates genetic diversity, helps study gene functions, and aids in developing organisms with beneficial traits, such as improved yield or disease resistance.

Chemical Mutagenesis:

Involves treating plants with chemical mutagens like Ethyl Methane Sulfonate (EMS), sulfur mustard, and nitrous acid to induce mutations.

The study by Xiu et al. (2020) investigates the impact of Ethyl Methane Sulfonate (EMS) mutagenesis on the genetic variability of Solanum melongena L., commonly known as eggplant. By inducing mutations, the researchers aimed to enhance genetic diversity and examine the phenotypic variations in the M2 generation of mutant eggplants, with a specific focus on anthocyanin content—a key pigment responsible for the purple coloration in eggplant fruit peel.

The study analyzed anthocyanin content across several M2 eggplant families, comparing the mutant strains to wild-type (WT) eggplants. Notably, the purple-black mutant (S9-1) exhibited a significantly higher anthocyanin content than the WT eggplants, indicating a successful enhancement of this trait through EMS mutagenesis. Conversely, the green mutant (L6-4) displayed the lowest anthocyanin content, registering only 17.27% of that found in the WT, while the white mutant (U36-1) also showed reduced anthocyanin levels.

The research further explored the molecular mechanisms underlying these phenotypic changes by analyzing the expression levels of anthocyanin biosynthetic and regulatory genes in the fruit peels of the mutants using RT-PCR. The results revealed that in the purple-black mutant (S9-1), there was a significant upregulation of several key anthocyanin biosynthetic genes, including SmCHI, SmDFR, SmF3H, SmF35H, SmANS, and SmCHS, compared to the WT. Interestingly, the expression of SmPAL did not increase, suggesting a more complex regulatory mechanism at play. In contrast, the green (L6-4) and white (U36-1) mutants exhibited a significant downregulation of these genes, correlating with their reduced anthocyanin content.

The M2 generation of EMS-mutagenized eggplants demonstrated considerable phenotypic diversity, particularly in terms of anthocyanin content, which is crucial for breeding programs aimed at enhancing desirable traits. The significant variation in anthocyanin concentration among the mutants, coupled with the corresponding changes in gene expression, highlights the potential of EMS mutagenesis as a powerful tool for exploring molecular genetic mechanisms and for the development of eggplant varieties with improved nutritional and commercial value. The study underscores the promise of utilizing mutagenesis in plant breeding to achieve specific trait enhancements, paving the way for future research and cultivation of superior eggplant strains.

Physical Mutagenesis

Physical mutagenesis involves using physical agents, such as radiation, to induce mutations in an organism's genetic material. This process primarily employs electromagnetic radiation, including gamma rays, X-rays, and UV light, as well as particle radiation such as fast and thermal neutrons, β (beta) particles, and α (alpha) particles. These radiation types can cause significant alterations in DNA, resulting in mutations. Exposure to these mutagens can increase the natural mutation rate by a factor of 1,000 to 1 million, making physical mutagenesis a powerful tool for generating genetic diversity and studying the effects of genetic changes in research and breeding programs (Tatomir and Koch, 2021).

Swathy *et al*. (2016) explored the effects of Helium-Neon (He-Ne) laser irradiation on the chlorogenic acid content and growth of brinjal (Solanum melongena L.) var. Mattu Gulla. The study found that laser treatment significantly enhanced seed germination and growth, with the highest germination rate observed at a 30 J/cm^2 dose. Chlorogenic acid content was highest in fruits from seeds treated with 20 J/cm^2. The research suggests that He-Ne laser irradiation is an effective method for improving both agronomical traits and biochemical properties in brinjal, highlighting its potential use in crop enhancement.

Sikder *et al*. (2019) investigated the effects of gamma irradiation and Ethyl Methane Sulfonate (EMS) on the tomato cultivar Berika. Seeds were exposed to gamma rays at doses of 50, 100, 150, 200, and 250 Gy. The study found that 50 Gy resulted in the highest germination rate (66.40%), tallest seedlings (13.25 cm), and greatest pollen fertility (78.58%). As the gamma ray dose increased, these traits generally declined, with the highest doses (200 and 250 Gy) leading to significant reductions in germination, growth, and reproductive success. Mutation frequency was highest at 50 Gy (4.39%), indicating that lower doses of gamma radiation are more effective for inducing viable mutations in tomatoes.

Insertional Mutagenesis

Insertional mutagenesis is a genetic technique where a segment of DNA is inserted into an organism's genome, disrupting the normal gene sequence. This disruption can cause mutations by interfering with or altering the function of genes at the insertion site. The inserted DNA may come from various sources, such as transposable elements, viral vectors, or engineered constructs. This method is extensively used in research to investigate gene function, identify essential genes, and develop models with specific traits in animals or plants. By causing gene disruptions, scientists can study the resulting phenotypic changes to gain insights into gene functions and their roles in biological processes.

Insertional mutagenesis is a genetic technique where a DNA segment is inserted into an organism's genome, disrupting the normal gene sequence and potentially causing mutations. The inserted DNA can come from various sources, including transposable elements, viral vectors, or engineered constructs. This method is valuable for studying gene function, identifying essential genes, and creating models with specific traits in both plants and animals. It also aids in identifying potential targets for genetic therapies, particularly for diseases related to gene dysfunction. However, insertional mutagenesis can sometimes lead to unintended effects, such as the activation of oncogenes, necessitating careful control and analysis (Singh and Prasad, 2014).

Mutagenesis by Antisense Approach

The antisense approach is a molecular technique used to selectively reduce or silence specific genes. It involves introducing antisense RNA or DNA sequences complementary to the messenger RNA (mRNA) of the target gene. These antisense sequences bind to the mRNA, blocking protein production or triggering mRNA degradation, leading to a functional "knockdown" of the gene. This technique allows researchers to investigate the effects of reduced gene activity and understand the gene's role in various biological processes (Kurreck, 2008).

Antisense Mutagenesis

The antisense approach involves introducing RNA or DNA sequences complementary to a target gene's mRNA to reduce or silence gene expression. This method is especially useful in functional genomics for studying gene functions and is being explored as a treatment for diseases caused by gene overexpression, such as certain cancers and viral infections. Unlike traditional mutagenesis, which often results in random mutations, the antisense approach offers a targeted and reversible way to modulate gene expression. However, it faces challenges like efficient delivery and achieving complete gene silencing. Despite these issues, it remains a valuable tool in research and gene-based therapies.

Genome Editing Approaches

Genome editing involves precise alterations of an organism's DNA to induce specific mutations. Techniques like CRISPR-Cas9, TALENs, and ZFNs enable targeted modifications, allowing researchers to create specific mutations for studying gene functions, modeling diseases, or developing new therapies. These tools introduce double-strand breaks in DNA, which are repaired by the cell's mechanisms, leading to desired genetic changes. While genome editing offers unprecedented precision and applications in agriculture and medicine, it also raises concerns about off-target effects and ethical issues.

Mutation Mapping Approaches

Mutation mapping identifies specific genomic locations responsible for particular traits or diseases. Classical methods include linkage analysis, while modern techniques such as genome-wide association studies (GWAS) and next-generation sequencing (NGS) offer comprehensive and precise mutation identification. Mutation mapping helps understand genetic variations, discover new genes, and develop targeted therapies. Notable applications include improving crop varieties and diagnosing genetic disorders. The concept of Mutmap, initially reported in rice, demonstrates the utility of mutation mapping in identifying genetic changes related to specific traits.

Mengnan *et al*. (2019) significantly advance cucumber genetics with their identification of the glabrous2 (gl2) mutant, which shows a notable absence of tubercles and spines on the fruit peel and a reduction in trichome density on stems and leaves. Discovered in an M2 population derived from ethyl methane sulfonate (EMS)-mutagenized cucumber inbred line 406, the gl2 phenotype is recessive. The study demonstrates that while F1 progeny exhibit wild-type characteristics, the glabrous trait appears in 23 out of 220 plants in the F2 generation. Crucially, genome sequencing and linkage analysis pinpoint a non-synonymous mutation in the Csa1G056960 gene as responsible for the gl2 phenotype. SNP analysis further corroborates this, showing homozygosity in the M-pool and heterozygosity in the N-pool. This research not only deepens our understanding of cucumber fruit and leaf morphology but also offers valuable insights for breeding programs focused on enhancing commercial traits.

MutChromSeq Approach:

This recently developed technique accelerates the detection of causal mutations by combining chemical mutagenesis with chromosome flow sorting. This method isolates specific chromosome regions to identify the gene of interest. It was first applied in barley and wheat, as reported by Sánchez-Martín *et al*. (2016).

iTILLING (Individualized TILLING):

iTILLING is an advanced version of the traditional TILLING method that cuts down both the cost and time required for mutation screening. It was specifically developed for use in Arabidopsis.

DeTILLING:

DeTILLING is a novel knockout technique designed to identify deletion mutants in target genes. It involves screening for rare deletion mutants within large populations subjected to fast neutron mutagenesis. Developed initially for Arabidopsis and rice, DeTILLING combines fast neutron mutagenesis with high-throughput PCR screening, also known as "Deleteagene."

Yong *et al*. (2021) explored the mutagenic effects of ethyl methanesulfonate (EMS) on nine protein-coding genes in tomato (*Solanum lycopersicum L.*), using a 0.5% EMS concentration to induce mutations in the ‘Improved Apollo’ cultivar. By selecting mutants based on phenotypic variation and sequencing nine specific genes—NRP2 (RNA polymerase II), HKX (hexokinase), P2D1 (photosystem II D1), NEXP (expansin), PHYSN (phytoene synthase), PCANH (carbonic anhydrase), NAGO (argonaute), PRUB (RuBisCO), and NGAG (gag-pol)—the study assessed genetic diversity and validated gene functions through ISSR fingerprinting and TILLING. The results highlighted diverse mutant phenotypes and confirmed the stable transmission of mutations from the M1 to the M2 generation, providing insights into the targeted gene functions and heritable changes in the mutant phenotype.

Table 1. NOVEL VARIANTS DEVELOPED THROUGH MUTATION BREEDING

Crop name	Mutant	Development details	Improved character
Potato	Nahita	Gamma ray @ 25 Gy on seed potato of cv. Marfona in 2016 at Turkey	Early maturity
	White Baron	From in-vitro culture of cv. Dansyaku at Japan.	Tuber tolerant to browning
	Kufri Sindhuri	Gamma rays (30-50Gy)	Adventitious bud regeneration /Tuber colour mutants
Capsicum & Chilli	Horgoska slatka-X-3	Gamma ray 3	Resistant to CMV
	Albena	135 Gy gamma rays, dry seeds, 19/0, [Zlaten medal]	More attractive fruits, better flavour because of lack of anthocyanin
	Orange Beauty	X-rays @ 120Gy on seed of F1 (Orangeva kapia x Duet)	High β- carotene, High content of Vitamin C, Tolerance to Verticillium decease.
	MDU1	Gamma rays	Compact plant type, higher yield and caspsaicine content
Brinjal	Daijiro	From in-vitro culture of original cv. Hirasu at Japan	Bacterial wilt resistance, high yield and resistance to low temperatures
	PKM 1	Gamma induced mutant of a local type called 'Puzhuthi kathiri' at Periyakulam, TN.	High yield, tolerance to drought, suitable for transport and storage to room temperature
Cassava	S-14 and S-15	Somatic embryos-rays (50Gy)	Embryogenesis Morphological mutants; mutants with storage root yield, altered cyanogens
Sweet potato	CO-3	Embryogenic suspensions- rays (80Gy)	Embryogenesis Mutants for salt tolerance
Tomato	Lanka Cherry	Gamma ray @ 320 Gy on seed of original cv. Manik in 2015	Easily distinguishable pear shaped fruits
	Magine	Gamma irradiation @ 300Gy on seed of cv. INCA 9-1 in 2017	Tolerance to drought and good quality
	PKM-1	Induced mutant from a local variety Annanji	Fruit weight 60 - 70g, Suitable for long distance transport. Yield 32-35 t/ha and duration 125 days.
Onion	Tabys (KIK-13)	With application of NEU @ 0.05% on original cv. October.	High yielder and directly used as induced mutant
	Brunette	With application of X-ray @ 150 Gy on seed of original cv. Grobol.	Very early maturity, combined with high yield and quality
Okra	Anjitha	Mutagenic treatment of breeding material on plant material of (A. esculentum var. Kiran × A. manihot) in 2016 at KAU.	General yield but resistant to viral disease and good quality
Okra	MDU-1	Application with dES @ 0.04% on parent material of Pusa Sawani in TNAU, Coimbatore.	Attractive light green long fruits, higher yield, fruit size, less crude fibre, field tolerant to yellow mosaic virus.
Bitter gourd	MDU-1	With application of gamma rays @ 100 Gy on original cv. MC 103	Resistance to insects (pumpkin beetle, fruit fly), leaf spot diseases, fruits dark, green with shallow groove..

Chinese garlic	Ningsuan 1	With application of gamma rays @ 15Gy on original cv. landrace	High yield, good quality, drought tolerance and diseases resistance
Chinese cabbage	Longfuer-niuxin	With application of gamma rays @ 700 Gy on original cv. Xinnongerniuxin	High yield, good flavour quality, resistance to bacterial diseases, resistance to soft rot, to downy mildew and to virus diseases.
Pea	Pusa-408 (Ajay)	Pisum sativum seed is exposed to sodium azide for mutation induction for development of nitrogen fixation mutants.	
Snake gourd	PKM-1	It is a induced mutant from H375	Yield potential of 25t/ha in 135-140 days.
Cucumber	Xianghuanggua 5	Gamma induced mutant in 2020 at Inst. of Horticulture Crops, Hunan AAS, China	Good fruit appearance, good quality, disease resistance and high yield.
	Altaj	With application of DMS @ 0.05% on seed of original cv. Altajskii.	Early maturity, high trade mark, tolerance to salinity and cultivation on open fields.

Advantages of mutation breeding

- Mutation breeding is a cheap and rapid method of developing new varities
- Induced mutagenesis is used for the induction of CMS and Ethidium bromide is used for induction of CMS
- Mutation breeding is more effective for the improvement of oligogenic characters
- Mutation breeding is the simple, easy and quick way to induce a new character

Limitations

1. Frequency of desirable mutations is very low about 0.1 percent. To detect the desirable one in M2 considerable time, labour & other resources are to be employed.
2. To screen large population, efficient quick and inexpensive

selection techniques are needed.

3. Desirable mutations may be associated with undesirable side effects due to other mutations thus extending the mutation breeding programme.
4. Detection of recessive mutations in polyploids and clones is difficult and larger doses of mutagen have to be applied and larger populations are to be grown.

Table 2. Works of Mutation breeding in Dharwad

Sl.no	Title	Author
1	Induced genetic variation for days to flowering and maturity and mutagenesis in chilli	S. Rangaiah 2006
2	Genetic variability and mutational studies in cluster bean	Swathi and T.R. Shashidhar, 2016
3	Studies on induced mutagenesis *invitro* regeneration in turmeric	R.V. Hegde and A.N. Mokashi, 2006
4	Induction of mutation by EMS and its effect on growth and flowering of chrysanthemum	Raveena *et al*., 2018

5	Estimation of chlorophyll and viable mutation under M2 generation in Rice Bean	Prakash and Shambulingappa, 1999
6	Comparative evaluation mutation and genetic variability in safflower	Veena and Ravikumar, 1999
7	Developmental of non lodging and early maturing linseed genotypes through induced mutagenesis	Suna *et al*., 2016
8	Estimation of LD50 for EMS treated population of groundnut	Avinash *et al*., 2018
9	Mutagenic effect of EMS on growth and yield components of M1 generation in Sorghum	Niveditha and Sajjanar, 2021

Table 3. Other Research Work On Mutation Breeding In World

Cocoyam and Taro: For root rot and leaf blight resistance in Ghana, Costa-Rica
For **improving selected grain cultivars** adapted to local conditions to increase **both quantity and quality of seed production** and to develop suitable varieties for mountainous marginal areas of Central Slovakia for income generation.
To develop improved varieties with **low solanine and solasodine content** in **bitter Andean potatoes.**
Induced mutation using gamma radiation to develop **resistant varieties** was selected as a viable option to breeding, in view of the fact that okra has always received modest research investment, particularly in Thailand

CONCLUSION

Mutation is a crucial strategy for expanding genetic variation and diversity in crops, helping to overcome genetic bottlenecks. Induced mutagenesis plays a significant role in unlocking the potential of plant genetic resources, enabling the development of new crop varieties with enhanced traits. These varieties, resulting from mutation breeding, substantially contribute to global food and nutritional security, and improve livelihoods by providing more resilient and productive agricultural options

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