Journal of Applied Biology & Biotechnology Vol. 9(04), pp. 162-169, July, 2021
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2021.9422

Improvement in ornamental, medicinal, and aromatic plants through induced mutation

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ARTICLE INFO

Article history:
Received on: December 21, 2020
Accepted on: February 22, 2021
Available online: July 10, 2021

Key words:
Mutagenesis, medicinal plants, mutagens, plant breeding, EMS, γ-rays

ABSTRACT

In several crop breeding programs, high selection pressure has been applied since its domestication which resulted in narrowing in the genetic variability. Therefore, obtaining new crop cultivars has become a difficult task for breeders. Development of strategies to increase the genetic variability has now become the prime area of research in crop breeding for several research groups. Mutation breeding is able to create lot of genetic diversity in the crops naturally as well as through induced mutagenesis. Mutation breeding is an important tool in plant breeding which has proven highly successful in improving crop varieties globally to feed an ever increasing and nutritionally demanding human population. Moreover, it has the advantage of improving a defective, but an elite cultivar, for a single trait without significantly affecting its acceptable phenotype. Till date, induced mutagenesis has been used in several crops including cereals, pulses, flowering plant, medicinal and aromatic plants for their many physical and biochemical trait improvement. Several physical and chemical mutagens like ethyl methane sulfonate, X-rays, or γ-rays have proven their importance in plant mutation breeding. After mutagenesis, selected superior mutants are recommended for large-scale production or further breeding in similar or different environments. Mutation breeding has been successfully applied in release of nearly 3,000 plant varieties especially in cereals, pulses, and flower and has a limited role in improvement of medicinal and aromatic plants. This review discusses the prospective effects of induced mutation in many medicinal and aromatic plants using different mutagens for their improvement in both quantitative and qualitative traits.

1. INTRODUCTION

Plant breeding is an important science for the improvement of all types of agricultural crops which has begun in very ancient along with the human culture. It mainly uses techniques like selection, hybridization, cross-breeding, mutation breeding, and even development of transgenic plants. Among all techniques, mutation breeding has become popular among the breeders and scientists as it can create genetic variation in plants naturally as well as through mutagens (physical or chemical). Although the first spontaneous mutant plants in cereal crops were found in China nearly 2,300 years before and even reported in 1,590 and 1968 by some scientist, the first publication on induced mutation was published by Muller [1] and Stadler [2–3]. The first commercial mutant cultivar was produced in *Nicotiana tabacum* using inducing mutations. After this event, mutation breeding has found a niche in plant breeding because of several advantages and has been applied actively to quickly adopt advances in the technology [4,5]. The Food and Agriculture Organization of the United Nations/ International Atomic Energy Agency-Mutant Variety Database data [6] reported that nearly 3,364 accessions from 226 species have been developed and officially released as mutants (Fig. 1). Some of these mutant cultivars have revolutionized agriculture not only in densely populated developing countries but also in agriculturally advanced countries like the United States, United Kingdom, Japan, France, etc. (Fig. 2). As per available data, India has also released nearly 344 varieties of different crops (Fig. 2).

Several studies have proved that induced mutagenesis is able to modify many morphological and biochemical plant traits like plant height, maturity, seed shattering resistance, disease resistance, oil quality and quantity, malting quality, size and quality of starch granules in many crops [7,8]. This mutagenesis has also become an alternative tool to develop resistance against harmful

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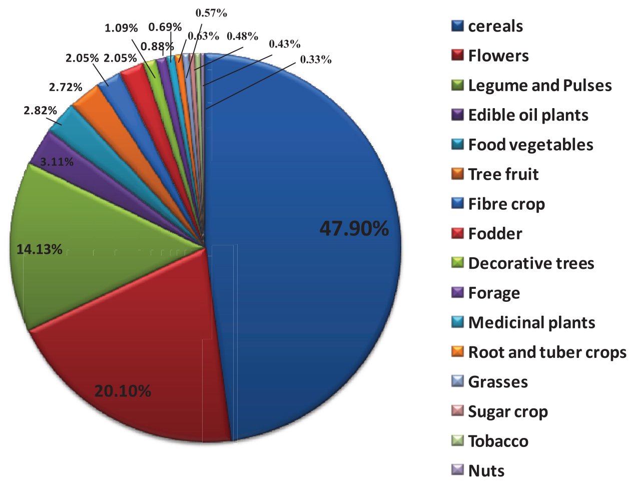

Figure 1: The Pie chart representing distribution of officially released mutants from different plant categories from all over world. The Mutant Variety Database contains more 3,364 entries. The above grouping is based on the plant type group. The top classes consist of cereals, flowering plant, and legume/pulses. Data collected from MVD 2021 (https://mvd.iaea.org/#!Search) accessed on Jan 20, 2021.

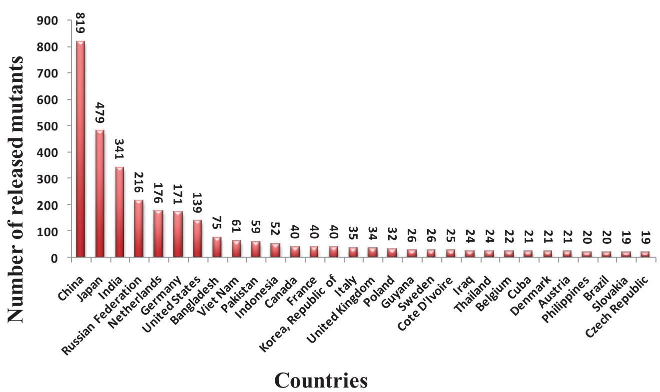

Figure 2: Country wise distribution of officially released mutants. Only top 30 countries were taken to make this bar diagram. Data collected from MVD 2021 (https://mvd.iaea.org/#!Search) accessed on Jan 20, 2021.

pathogens in many susceptible crops, e.g., ginger and coriander, to their improvement in yield and quality traits [9] and (Table 1). It is well known that transgenic technology has been significantly used in improving crops with substantial commercial value, but this technology has some technical limitations in many crops as these plants are highly recalcitrant to genetic transformation and regeneration. Further, the other important method of improvement is the development of transgenic crops but this technology is very much doubtful for the unpredictable risk of environment and food safety [10]. For these kinds of problems, mutagenesis can be used without restrictions like legislative constraints, licensing costs, and societal opposition of transgenic technology [11]. Mutagenesis with high-resolution screening can be able to supply a very good complement to recombinant DNA technologies for the improvement of crops for better adoption in changing environment conditions for increasing global population [12]. Mutants are now effectively used for studying gene expression and gene regulation in many crops and also able to assign a functional role to genes. Induced mutations enhance the rate of genetic variability, introduce many new traits, and identify trait specific genes [13]. Mutation breeding has been proved as a supplement and an effective substitute to conventional breeding where only specific improvement in a variety is required without losing its original acceptable phenotype.

1.1. Types of Mutagens Used in Mutation Breeding

Mutations are responsible for creating genetic variability in several crops naturally or artificially induced. Several physical and chemical mutagens are being used to induce artificial mutations [14,15]. Physical mutagens include various types of radiations like X-rays, gamma rays, neutrons, beta particles, alpha particles, proton or deuterons, UV rays, etc. (Fig. 3). The chemical mutagenesis is caused by several chemicals like alkylating agents [ethyl methane sulfonate (EMS), Methyl methanesulfonate, nitrogen mustard, etc..], base analogues [Bromo uracil (5BU), aminopurine (2AP), Tetramethyl Urea (TMU), etc..], Acridine dyes (Acriflavine, acridine orange, ethidium bromide, etc.). Another category for tool for mutagenesis is the use of biological agents such as transposons, retrotransposons, and T-DNA [16,17]. The genome editing systems like transcription activator-like effector nucleases (TALENS) and clustered regularly interspaced short palindromic repeat (CRISPR) induce target mutations and can be used as an alternative mutagen [18] (Fig. 3). These chemicals have chromosome damaging effects on plants by oxygen derived radicals; these effects can occur both spontaneously and artificially following induction through mutagens [9]. There are several mutagens available for crop improvement and each has its own important role as positive or negative effect on crops [19].

1.2. Steps Involved in Mutation Breeding

Several steps are involved in the process of induced mutagenesis (Fig. 4). The first step involves irradiation or chemical treatment of the seeds or any tissue of the parent variety and this material is designated as M_0. These Mutagenic treatments can induce some chromosomal rearrangements or can make some changes in genes at the nucleotide level to develop other allelic forms. This alteration may be responsible for the change in one or more specific characters in the M_1 generation which is grown from mutagenized seed or tissue (M_0). The population of M_1 generation is homogenous type as the plants may have a different effect of allelic mutation. Genetically, M_1 mutant plants are heterozygous in nature as in most of the cases only one allele is affected by one mutation during treatment. The occurrence of having mutation in both alleles of a gene is extremely low and very rare. Moreover, only dominant mutations can be identified in M_1, while it is impossible to identify a recessive mutation. Therefore, the selection is not done in M_1 generation and plant breeders attempt screening mutations in subsequent generations where segregations occur. Now the seeds of M_1 generation are used to create M_2 generation [20]. The seeds of M_2 generation are sown in experimental plots to obtain segregating M_2 population which is subjected to various screening procedures for the selection of plants with desired characters. The selected plant material of M_2 is further grown on as

Table 1: List of improvements in medicinal and aromatic crops through Mutation breeding.

Crop	Mutagens used	Improved trait	References
Henbane (*H. niger* L.)	Irradiated culture	Vegetative growth, yield and alkaloid content	23
Black cumin (*N. sativa* L.)	X-rays and gamma radiations	Improve the many yield attributing characters	24,25
C. sativum	EMS induced mutation	Resistant to stem gall and less susceptible to the powdery mildew	22
C. roseus	Chemical mutagens	High alkaloid	27
C. roseus	Chemical mutagens	Leafless inflorescence architecture and increased flower frequency and salt tolerant	28
C. roseus	EMS induced mutation	Higher total root and leaf alkaloids including anticancer alkaloids, vincristine and vinblastine	29
Fenugreek (*T. foenum-graecum* L.)	EMS induced mutation	Determinate growth habit, early maturity and high seed yield	31
Fenugreek (*T. foenum-graecum* L.)	Gamma rays, EMS, and EI	Higher yield and diosgenin content	32
Opium (*P. somniferum)*	Gamma rays and EMS	Alkaloid free	33
Opium (*P. somniferum)*	EMS	Higher seed yield and high morphine	34
Thorn apple (*D. inoxia* Mill.)	EMS, DES, gamma rays either alone or in combination	Higher alkaloid scopolamine yield with altered agronomic performance	35
C. forskohlii	Gamma irradiation	Leaf shape, plant height, leaf color, tuber yield, and forskolin content	36
Isabgol (*P. ovata* L. Forsk)	Ionizing radiation	High seed yield	40–43
Chamomile	Gamma irradiation	Plant height, flower number, and oil yield	44,45
Peppermint (*M. piperita*)	Gamma rays	Higher oil yield constituents like menthol and menthone	22,46,47
Ginger	Gamma rays and EMS	Resistance against *R. solanacearum* and *Pythium* sp.	48
C. longa	Gamma rays and EMS	Curcumin content (up to 4.2%) in rhizomes and crop yields	22
C. martinii	Gamma irradiation	Higher yield and better quality	54
C. winterianus	X-rays irradiations	Genetic divergence and better oil composition	55
C. citratus	UltraViolet-B exposure gamma irradiation	Production of more oil cells change in the quality and percentage of essential oil contents and physical characteristic	56,57

the M_3 generation. Usually, $M_{3–5}$ generations are used to screen the quantitatively inherited characters, homozygosity, and stability in field performance on a line or a single plant basis. These selected promising homozygotic mutants can multiply directly in the field in subsequent generations $M_{6–8}$ and comparative analysis is also done with checks in different locations After the multiplication of seed and different location testing, the mutants can go for official testing before release as mutant variety (Fig. 4). Further, these lines can be used as breeding material in a cross breeding program [20,21].

1.3. Improvement in Medicinal and Aromatic Plants Using Mutagenesis

The conventional plant breeding methods are very effective in many crops including cereals and pulses and have been using since long to improve the yield and other characters in these crops. But these conventional breeding methods are not very much successful and take a long period for improvement in high yield of biomass and increase in the active principles in medicinal and aromatic crops due to low genetic variability. Hence, induced mutagenesis has been proved as an important approach for creating the genetic variation and diversity in these crops to overcome from these bottleneck conditions. Induced mutagenesis can utilize the potential of plant genetic resource to create variability which is available to plant breeders as raw material to generate new crop variety and these varieties can provide a significant contribution for sustainable crop production. The contribution of mutation breeding in medicinal and aromatic crops is less than one percent which is comparatively very less as compared to cereals (48%), flowers (22%), and legumes (15%) [22] (Fig. 1). There is scope for improvement in medicinal and aromatic crops through mutation breeding in terms of enhancement of important features like new colors and shapes of flower, plant morphology, flowering times, postharvest quality, biotic and abiotic stress tolerance, and alkaloid content in short time with low cost. The mutation breeding was successfully done in several spices, flower species, grasses, and wild species that come under medicinal and aromatic plants to improve several characters like yield, alkaloid contents, disease resistance, etc. (Table 1). In this review, some cases out of those several mutagenesis studies in medicinal and aromatic plants are discussed.

Farooqi and Shriramu [23] released a Henbane (*Hyoscyamus niger* L.) variety, Aela, belong to the family Solanaceae using irradiated culture. The developed variety had more than double vigorous growth with a yield of 73 q/ha as compared to its parent (50 q/ha), and also records a higher alkaloid level 0.545% as compared to 0.167% in the parent plant. This mutant can be identified by its yellow flowers with a slight purple tinge at the base of the petals. In black cumin (*Nigella sativa* L.), some superior mutants lines were developed by induced mutagenesis through X-rays and

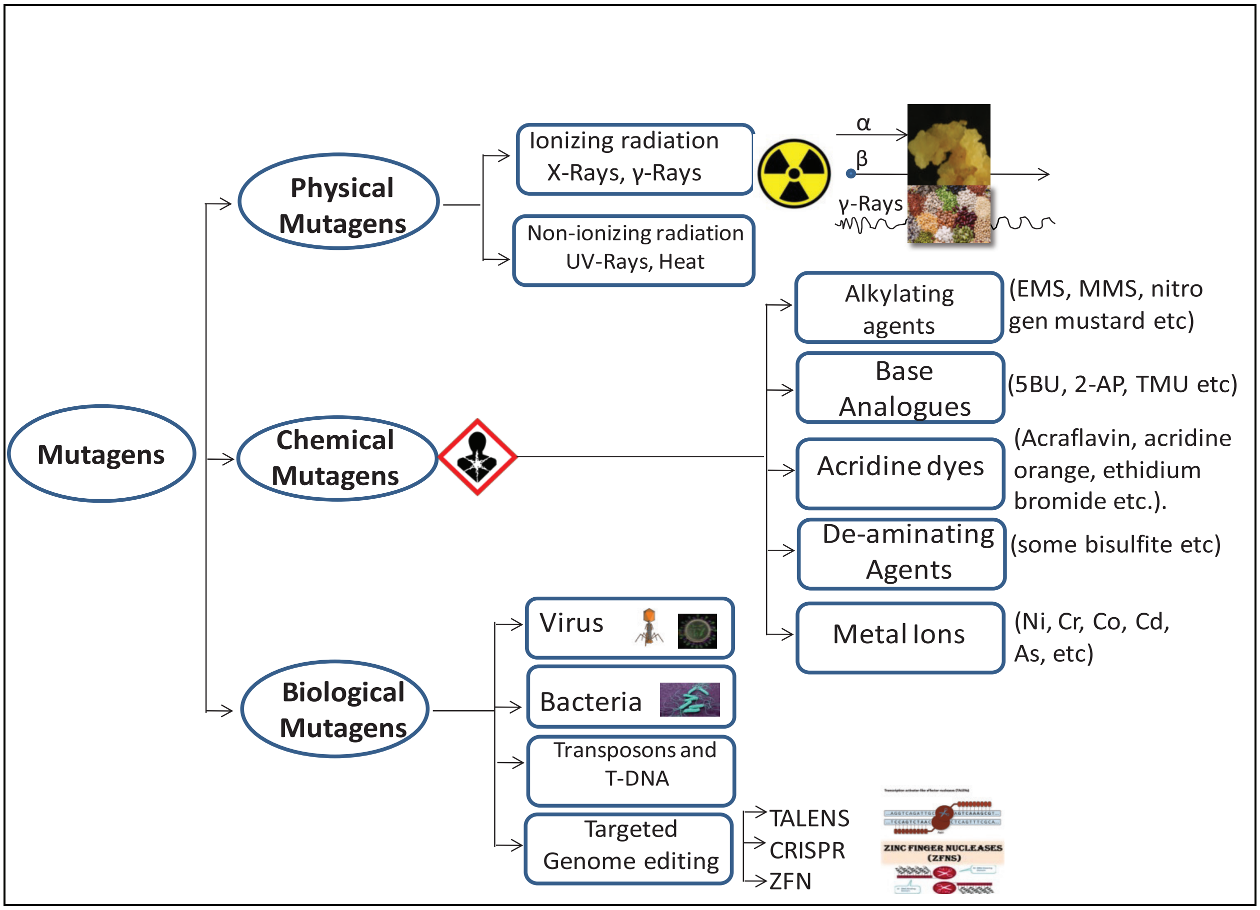

Figure 3: Diagram representing the three different kinds of mutagens used in mutagenesis of different crops. Abbreviations: EMS = Ethyl methyl sulphonate; MMS = Methyl methanesulfonate; 5BU = Bromo uracil; 2AP = aminopurine; TMU = Tetramethyl Urea; TALENS = transcription activator-like effector nucleases; CRISPR = clustered regularly interspaced short palindromic repeat; ZFN = Zinc Finger Nucleases.

gamma radiations and proper selection to improve the many yield attributing characters like lax branching, feathery leaf mutant, bushy, male sterile, crumpled leaf, early flowering, brown seed coat, etc. [24]. The gamma irradiation and EMS treatment on dry, filled, and black seeds of cumin were able to produce five dark reddish brown, one yellowish brown, and one peach color seeded plants out of 7,956 plants progenies [25]. A mutant line RZ-223 of *Cuminum cyminum* developed through mutation breeding using gamma rays was found to resistant to wilt and blight diseases, superior in yield and seed quality over its parental line [26]. In *Coriandrum sativum* a mutant variety "RCr 684" was developed for resistant to stem gall and less susceptible to the powdery mildew [22].

Kulkarni and Baskaran [27] used *Catharanthus* seeds for chemical mutagens treatment to develop a high alkaloid producing variety "Dhawal". Another *Catharanthus* mutant with leafless inflorescence, increased flower frequency, and salt tolerant was also developed using chemical mutagenesis [28]. A mutant variety of periwinkle with higher total root and leaf alkaloids including anticancer alkaloids, vincristine and vinblastine, was also developed through EMS induced mutation [29]. A dwarf mutant of *Catharanthus roseus* was developed to evaluate morphological parameters and the antibacterial activity of the aqueous and ethanol leaf extracts against five medically important bacterial strains [30].

Basu *et al*. [31] reported that the mutation breeding approach in Fenugreek (*Trigonella foenum-graecum* L.) seeds using different dose of EMS for different times and mutated plants were selected for new breeding material with several improved characters. In another report, several mutant lines were selected for higher yield and diosgenin content as compared to their parental lines based on morphological performance from M2 to M4 generation after treated with different mutagens like gamma rays, EMS, and ethylene imine (EI) with different dose and time [32]. The mutation breeding approach was also applied in narcotic opium to make non-narcotic and alkaloid free opium variety "Sujata" using gamma rays and EMS [33]. Five improved opium poppy mutant lines showing higher seed yield and high morphine content over control varieties of commercial values were developed from the progeny of a known poppy variety using gamma rays of additionally with EMS [34]. A variety of induced mutation using EMS, diethylsulfate (DES), gamma rays either alone or in combination was applied in Thorn apple (*Datura inoxia* Mill.) which resulted in development of several homozygous lines of the mutants with higher alkaloid scopolamine yield with altered agronomic performance [35].

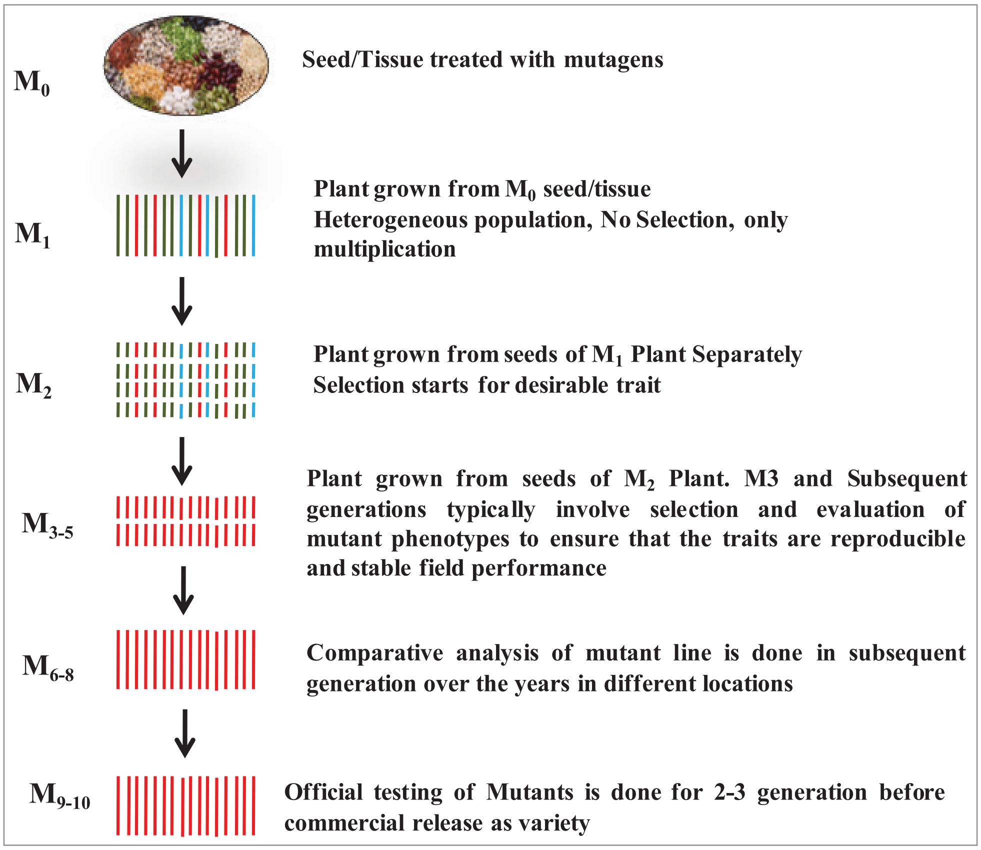

Figure 4: Flow chart of traditional mutation breeding scheme. Each row describes the steps for a specific generation. The generation nomenclature starts with M_0. Subsequent generations are designated as M_1, M_2, etc.

An important medicinal plant *Coleus forskohlii* member of Lamiaceae family grows wildly in the subtropical climate. The roots and leaves have been useful in the treatment of various allergic disease, viz., psoriasis, eczema, skin infections, leucorrhea, and asthma. The tuberous roots are found to be a rich source of the labdane diterpenoid forskolin (22). Srinivasappa *et al*. [36] used tissue cultured plant, root cuttings, and un-rooted cutting for gamma irradiation to improve various quantitative and qualitative traits like leaf shape, plant height, leaf color, tuber yield, and forskolin content. In another attempt, a variety Suphala was developed for high yield (15.93 t/ha) and can be cultivated throughout the year.

Solanum viarum is a perennial shrub with a prickly stem and prickly leaves which yields solasodine, an active ingredient of the contraceptive pill. A mutant Arka Sanjeevani a Spineless variety of *S. viarum* was also developed through mutation breeding for high alkaloid content [37]. The strains such as Glaxo BARC, RRL-20-2, and RRL-SL-6 were developed with very high yield potential for alkaloids through mutation breeding [38,39]. Isabgol (*Plantago ovata* L. Forsk) is an important indigenous drug plant of India. The seed and husk are used in ayurvedic medicines to treat many stomach disorders such as diarrhea, ulcers, constipation, and piles. Several investigators have worked on ionizing radiation for inducing mutation in *psyllium* and released some varieties like Niharika, Mayuri, and Nimisha for high seed yield up to 10–11 q/ha [40–43]. Mayuri was a distinct early maturing, high seed yielding potential of about 13 q/ha seeds of *psyllium* with the distinct pigment marker which can indicate the right stage of panicles maturity for harvesting to avoid the seed shattering in *psyllium*. In chamomile, two improved mutant lines were developed using gamma irradiation with changes in plant height, flower number, and oil yield in both positive and negative directions. These two lines also showed changes in some key enzymes responsible for the biosynthesis of quality components and subsequently two varieties, "Vallary" and Ujjawla, are released for commercial cultivation with Dry flower yield 7.0 q/ha and Oil yield 6.0 kg/ha [44,45]. Mutation breeding resulted in the development of another variety CIM-Sammohak with a higher yield of dry flowers (7.5 q/ha) and oil yield of 6–7 kg/ha containing important alkaloids like 12% chamazulene, 20% bisabolol oxide-A, and 11% bisabolol oxide-B (22).

A widely adapted peppermint (*Mentha piperita*) variety "Kukrail" and "Pranjal" with erect plant habit was developed for higher oil yield and disease resistant against *Spilarctia obliqua* (Bihar hairy caterpillar). Another peppermint variety "Tushar" has also been developed through mutation breeding for higher oil produce up to 85–90 kg/ha oil [22]. A high yielding and disease resistant (against *Puccinia menthae*) peppermint variety "multimentha" was also developed through induced mutation. This variety showed improved features like high essential oil content and resistance against pathogens [22,46]. Various studies showed that irradiation by gamma rays in *M. piperita* L. resulted in various mutations that may be used for the plant breeders to create new varieties of mentha. These mutated lines may be very useful for pharmaceutical companies and industries due to high essential oil constituents such as Menthol (48%) by the dose of 8 k-Rad gamma rays and Menthone (21%) by the dose of 6 k-Rad [47].

Improvement in the vegetatively propagated crops like ginger and turmeric can also be done using induced mutagenesis. In ginger, six potential mutants were screened for resistance against *Ralstonia solanacearum* and *Pythium* sp. after mutagenesis [48]. Other researchers were also found that the different doses of gamma rays and EMS can cause significant variations in the number of mother, primary, secondary, and tertiary rhizomes in ginger and turmeric [49–52]. In *Curcuma longa,* some varieties like BSR1 and BSR2 were also developed using mutagens for higher curcumin content (up to 4.2%) in rhizomes and crop yields up to 32 t/ha in a shorter crop duration of 240–250 days while another variety CO.1 has bold and orange yellow rhizomes, suitable for drought affected areas [22].

The improvement in genus *Cymbopogon* using mutagenesis has been carrying out since long. A methyl-eugenol deficient mutant of *Cymbopogon flexuosus* was developed using X-rays radiation which made it a good substitute for citronella oil [53]. Gamma irradiation on the seed of Palmarosa (*Cymbopogon martini*) resulted in a higher yield and better quality of the essential oil [54]. Several varieties of *Cymbopogon winterianus* like Bhanumati, Bibhuti, Niranjan, Phullara, Sourar, and Subir have been developed through mutation breeding with genetic divergence and better oil composition for different regions and climatic conditions at North Indian and South Indian [55]. CIM-Jeeva, a variety of *C. winterianus* with herb yield 215 q/ha and oil yield 285 kg/ha has also been developed through mutation [22]. Treatment of UltraViolet-B exposure was able to stimulate the production of oil cells in changing the quality and percentage of essential oil contents of lemongrass, i.e., *Cymbopogon citratus* [56]. Gamma irradiation was able to create changes in physical characteristic in *C. citratus* at the early growth phase in M1 generation [57]. Five improved mutant clones of Jamrosa were isolated with variation in quality/quantity of essential oil using various doses of gamma-rays in dormant vegetative slips [58].

1.4. Limitations and Challenges for Mutagenesis

As the other plant breeding methods, mutation breeding is also having some limitations. Among them, the most important is non-availability of sequence information of these medicinal plants like other cereals and pulses. These plants also have distinct characteristics such as the phylogenetic distance from other model plants. Therefore, both forward and reverse genetics are restricted in medicinal plants due to the unavailability of genomic. The gene information of many alkaloids and other essential biochemical information are very limited for medicinal plants. Regeneration and transformation methods of several medicinal plants are rarely explored. The development of gene editing and mapping approaches has enabled the progressions in the improvement of medicinal and aromatic plants. The implication of a combination of approaches of improved genomics and breeding methods can help to overcome the above-mentioned limitations towards mutagenesis research in medicinal and aromatic plants [59]. Successful application of mutagenesis on medicinal plants can be proved as a basic model system for the application and guidelines for future planning of induced mutagenesis in several other important and orphan crops for their improvement [60].

2. CONCLUSION

Mutational breeding is considered as an important and alternative approach to plant breeders for increasing crop productivity. Creation of variation and diversity in a given crop is the biggest advantage of mutation breeding. Mutagenesis coupled with *in vitro* selection and plant biotechnology approaches allow plant breeders to screen for characters that were tough to obtain in breeding. Therefore, the researchers are able to break yield plateau and enhance tolerance using variability by a new combination of genes created in mutagenesis. The superior lines developed through mutation breeding can be used directly as improved lines or can be used as breeding material for developing new population for improved varieties. These newly developed crop varieties through mutation breeding can significantly contribute to global food and nutritional security. Further, several agents for mutagenesis (target or random) are proven as revolutionary tools for plant breeding. The contribution of mutation breeding in medicinal and aromatic crops is less than the other cereals and pulse crops due to unavailability of genomic information and phylogenetic relationship with model plants. Therefore, there is huge scope for improvement in alkaloid contents, oil yield, and some other biochemical properties of medicinal and aromatic crops through mutation breeding. Considering these aspects, it can be said that mutagenesis certainly has a great impact on crop genetics and breeding in past, present, and future.

3. ACKNOWLEDGEMENTS

The authors are thankful for DBS, global, Dehradun for providing internal project funding and support for research work and publication.

4. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

5. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

6. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

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How to cite this article:
Agrawal L, Kumar M. Improvement in ornamental, medicinal, and aromatic plants through induced mutation. J Appl Biol Biotech 2021; 9(04):162–169.