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Role of mutation breeding in improvement of medicinal and aromatic crops: Review

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Abstract
Medicinal and aromatic plants are important sources of plant secondary metabolites, which are important for human health care. Over three-quarters of the world population relies mainly on plants and plant extracts for health care. More than 30 per cent of the entire plant species at one time or otherwise used for medicinal purposes. Variability in most of the medicinal and aromatic crops is very narrow. Mutation breeding has been successfully utilised for the improvement of crops as well as to supplement the efforts made using traditional methods of plant breeding. Induced mutation is the ultimate source to alter the genetics of crop plants that may be difficult to bring through cross breeding and other breeding procedures. Therefore, during the last several years, different mutagens have been used by various workers to induce genetic variability in various medicinal and aromatic crops. In periwinkle 'Dhawal' (NCIMB Accession No. 41147), a high alkaloid producing variety produced by chemical mutagen treatment of seeds followed by rigorous selection in widely cultivated variety 'Nirmal'. Variety Aela was developed in henbane with vigorous growth and yield of 73qt which was 109 per cent more than the normal variety. There was increase in the level of steroidal sapogenin (diosgenin) in *Trigonella corniculata* following chemical mutagenesis using dimethyl and diethyl sulphate. Variety Sujata was developed through mutagenesis in opium poppy which is non narcotic (opium less and alkaloid free). Thus mutation assisted plant breeding will play a crucial role in the generation of designer crop varieties to address the threats and challenges of present and future needs in medicinal and aromatic crops.

Keywords: mutation breeding, medicinal and aromatic crops

Introduction
Medicinal and aromatic plants (MAPs) are important sources of plant secondary metabolites, which are important for human health care (Kumar and Gupta, 2008) [1]. Exploiting natural or induced genetic diversity is a proven strategy in the improvement of all major crops, and the use of mutagenesis to create novel variation is particularly valuable in those crops with restricted genetic variability. Historically the use of mutagenesis in breeding has involved forward genetic screens and the selection of individual mutants with improved traits and their incorporation into breeding programmes (Parry *et al.*, 2009) [2].

Over three-quarters of the world population relies mainly on plants and plant extracts for health care. More than 30 per cent of the entire plant species at one time or otherwise used for medicinal purposes. Since ancient times, herbal medicine has played a major role in traditional systems of medicine such *Unani* and *Ayurveda*. It's very sorry state of the medicinal plant dependent industries that for production, they are totally dependent on the plant material that is collected from the natural habitat resulting in depletion or total loss of medicinal plant germplasm. Studies on medicinal plants are rapidly increasing because of the search for new active molecules, and for the improvement in the production of plants or molecules for the herbal pharmaceutical industries (Bhau, 2012) [3].

The basis for use of medicinal plants is that these plants contain so called active principles that affect physiological processes of living organisms. An aromatic plant indicates plants having sweet smelling or fragrant aroma. In modern ages, Medicinal and Aromatic Plants (MAP's) are defined as fragrant ingredient containing group of medicinal plants (Lakshman Chandra De, 2017) [4], The MAP sub-sector has immense potential as the sustainable commercialization can benefit farmers and industry both by providing higher price and by opening up national and global markets for new products from the region. Private sectors stand to benefit by ensuring sustainable supply of quality raw materials to benefit their industry and trade if they can be facilitated to build partnerships with farmers (Singh, 2009) [5].

Genetic improvement work without a wide variability in the population is not possible. Intraspecific variation are pre-requisite for initiating any plant improvement programme, which forms the basis for selection and improvement (Datta, 2005) [6]. The number of genes expressed during the lifetime of a particular plant is estimated to be between 16,000 and 33,000 (Gibbson and Somerville, 1993) [7]. Spontaneous and induced mutations in plants contribute for genetic dissection of the wild type genes (Datta, 2005) [6], Hugo de Vries (1901), a pioneer in the study of mutations, defined mutation as the sudden heritable change in the character /genetic material at the gene or chromosome level of an organism. The genetic information of all living organisms is located in discrete segment of the chromosome DNA termed as genes Mutations are very important as they from the entire genetic basis for the evolution of species in nature and the basis for the artificial development of new plant cultivars, as their mutation is ultimate source of new genes. The term mutation breeding was first coined by Freisleben and Lein, 1944 to refer to the deliberate induction and development of mutant lines for crop improvement (Shu *et al.,* 2012) [8].

Mutagenesis is an important tool in crop improvement and is free of the regulatory restrictions imposed on genetically modified organisms. The forward genetic approach enables the identification of improved or novel phenotypes that can be exploited in conventional breeding programmes. Powerful reverse genetic strategies that allow the detection of induced point mutations in individuals of the mutagenized populations can address the major challenge of linking sequence information to the biological function of genes and can also identify novel variation for plant breeding (Parry *et al.,* 2009) [2].

Medicinal plants are used for their active principles that influence the physiological processes of an organism; mutation gives rise to a variant with secondary metabolic profile called as chemical races or chrmodemes (Lakshman Chandra De, 2017) [4]. The widespread use of induced mutants in plant breeding programme across the globe has led to the official release of 3222 plant mutant varieties from 170 different plant species in more than 60 countries throughout the world (Shu, 2009) [9]. The developed varieties increase biodiversity and provide breeding material for conventional plant breeding thus directly contributing to the conservation and use of plant genetic resource (Raina *et al.*, 2016) [10]. Currently, according to the IAEA (2007), China ranks first with a number of 638 radiation-induced varieties, followed by India with 272 varieties, and Japan with 233 varieties (Nakagawa, 2009) [11].

Medicinal and Aromatic Plants (MAP) are known for their active ingredients as secondary metabolites and fragrance compounds at lower concentration in plant system. Breeding objectives' of MAP's includes high and stable yield, high content of desire active compounds, resistance to insect pests and disease as well, tolerant to abiotic stress like moisture stress, high temperature, soil salinity and alkalinity, and absence of harmful substances and also wider adaptability. The potential of induced mutation in plant breeding has long been established.

Types of mutagens

These include both physical and chemical mutagens, where in physical mutagens include various types of radiations like x-rays, gamma rays, neutrons, beta particles, alpha particles, proton or deuterons, UV rays *etc*., in recent times, chemical mutagenesis *viz.*, Alkylating agents (EMS, MMS, nitrogen mustard etc), base analogues (5BU, 2-AP, TMU *etc*), Acridine dyes (Acraflavin, acridine orange, ethidium bromide *etc.*) has become an important tool in crop improvement. These mutagens are being used to produce resistance in various susceptible crops to improve yield and quality traits against harmful pathogens. there are several mutagens available for crop improvement and each has its important role as positive or negative effect on crops (Fahad and Khan, 2001) [12], many of these chemicals have chromosome damaging effects on plants by oxygen derived radicals these effects can occur both spontaneously and artificially following induction through mutagens (Sharma and Sharma,2014) [13].

Steps in mutation breeding

The general procedures for using induced mutations are rather simple and have a strong basis in the laws of genetics (FAO/IAEA, 1977). Dormant seeds of the so-called parent variety are irradiated or treated with a chemical mutagen. Mutagenic treatment can cause chromosomal rearrangements or change some genes to other allelic forms. Plants grown from mutagenized seeds are called M_1-plants. When a multicellular tissue like the seed embryo is treated with a mutagen, the plant developing from the treated seed has a chimeric structure from a genetic point of view. After meiosis, the seeds developed on M1 plants are already the M_2 generation. These seeds are sown in experimental plots, and a segregating M_2 population is subjected to various screening procedures for desired characters. Selected material is then usually grown on as the M_3 generation. Screening for quantitatively inherited characters is usually done in the M_3 generation where selection on a line, rather than on a single plant basis, can be initiated. Selected mutants from the M_2 or M_3 generations are usually checked for homozygosity in the M_3 or M_4 generations, respectively. Promising, homozygotic mutants can be used directly for multiplication - this will lead to the development of the so-called direct mutant variety or they can be used in a cross breeding programme (Maluszynski, 1995) [14].

In a comparative study on effect of different dose/concentration of gamma rays, ethyl methanesulfonate (EMS) and sodium azide (SA) on various biological parameters (seed germination, seedling height, plant survival, pollen fertility and chromosomal aberrations Mutagenic effectiveness decreased with the increase in dose/concentration of the mutagen where as the efficiency of mutagens showed variable trend depending on the criteria selected for its calculation. The lower or intermediate treatments of all the mutagens were found more efficient in causing less biological damage and inducing maximum macro-mutations. The order of mutagenic efficiency was EMS > SA > ϒ- rays (Bhashir *et al.* 2013) [15].

Achievements in Medicinal and Aromatic plants through mutation breeding

Achievements through mutation breeding in medicinal and aromatic crops are very less when compare to the cereals (49.50%), flowers (21.9%) and legumes (15.0%). In medicinal and aromatic crops (0.20%) varieties have been released (Fig 1) but still there is scope for improvement in medicinal and aromatic crops through mutation breeding as in these crops enhancement of alkaloid is prime important feature than the total crop yield.

Henbane (*Hyoscyamus niger* L., 2n=34) belong to the family solanaceae, a variety Aela has been released from the CIMAP, Lucknow, which was selected from irradiated culture of *H. Niger*, having vigourous growth with a yield of 73q/ha, which was109 percent more than the parent (50q/ha), with the alkaloid level 0.545 per cent against 0.167 per cent in the parent plant. This mutant can be identified by its yellow flowers with slight purple tinge at the base of the petals (Farooqi and Shriramu, 2001) [16].

In black cumin (*Nigella sativa* L.) mutants like lax branching, feathery leaf mutant, bushy, male sterile, crumpled leaf, early flowering, brown seed coat, dwarf and viridis mutants were developed through X-rays and gamma radiations which were superior for many yield attributing characters stating that the parental genotypes and cross combinations identified from induced mutant progenies offer scope of improvement through proper selection in black cumin (Datta, 2005) [5].

Kulkarni and Baskaran *(*2003) [17] reported a periwinkle variety 'Dhawal'(NCIMB Accession No. 41147), a high alkaloid producing variety produced by chemical mutagen treatment of seeds followed by rigorous selection in widely cultivated variety 'Nirmal'.

Kumar *et al.*, (2007) [18] reported a unique inflorescence bearing mutant (lli / lli) which was a monogenic recessive having leafless inflorescence architecture with increased flower frequency. This distinct plant of *C. roseus* was developed through chemical mutagenesis followed by salt tolerance selection (US Patent PP18315). Baskaran *et al.*, (2013) [19] reported two EMS induced macro-mutants ('necrotic leaf'and 'nerium leaf) of periwinkle with enhanced contents of total root and leaf alkaloids and anticancer leaf alkaloids, vincristine and vinblastine than the parental variety. Their leaf and root yields were, however, significantly lower than their parental variety.

Basu *et al.*, (2008) [20] reported that mutation breeding approach has detected new breeding material exhibiting early seed maturity coupled with high seed yield, seed quality and determinate growth habit, when Seeds from Tristar Fenugreek, were treated with 10–300 mM ethyl methane sulfonate (EMS) for 2–24 h and plants were selected for determinate growth habit, early maturity and high seed yield. Seeds of fenugreek (*Trigonella foenum-graecum* L.) were treated with either gamma rays (25-15 kR) or ethyl methanesulphonate (EMS 0.1-0.5%, 2h) or ethylene imine (EI, 0.01- 0.1%, 2h). After repeated selection for better morphological performance from M_2 to M_4 generation, 11 mutant lines were identified with better yield potential and higher diosgenin content than their parent (Floria and Ichim,2006) [21].

A mutation breeding programme using gamma rays and ethyl methane sulphonate (EMS) was carried out for genetic conversion of narcotic 'opium poppy' into non- narcotic 'seed poppy', The best mutant genotype, LL- 34 of the family C^1- Comb- 113- 2, with 5.66 g/capsule seeds containing 52.6 per cent oil was designated as cv. 'Sujata'. This, perhaps the world's first opiumless and alkaloid- free seed poppy cultivar, offers a cheap and permanent (fundamental) solution to the global problem of opium- linked social abuse (Sharma *et al.*, 2008) [22]. Five opium poppy mutant lines were selected from the progeny of the poppy variety, De Botosani, after treatment with either gamma rays of additionally with EMS. Most of the lines showed significant increase of both seed yield and morphine content over commercial varieties in two locations (Floria and Ichim, 2006) [23].

Torn apple (*Datura innoxia* Mill.) was treated with methanesulphonate (EMS), diethylsulfate (DES), gamma rays, singularly or in combination. From a mutant with purple-red stem colour, petiole and foliar nervures identified in M_2, several homozygous lines were developed in M_5, which showed altered agronomic performance and increased alkaloid yield (scopdamine). The results suggested induced mutations could be used for genetic improvement of torn apple (Floria, 2006) [24].

Further high yielding peppermint variety – multimentha: which has a high essential oil content and resistance against Puccinia menthae., Chamomilla recutita (zloty lan Bdegold), Origanum majorana (Miraz, Tetreta), Verbascum phlomoides-Polyveb and Lavandulavera (Slavova *et at.*, 2004) [25] have been developed. Some of the other varities developed through mutation breeding are listed in Table1.

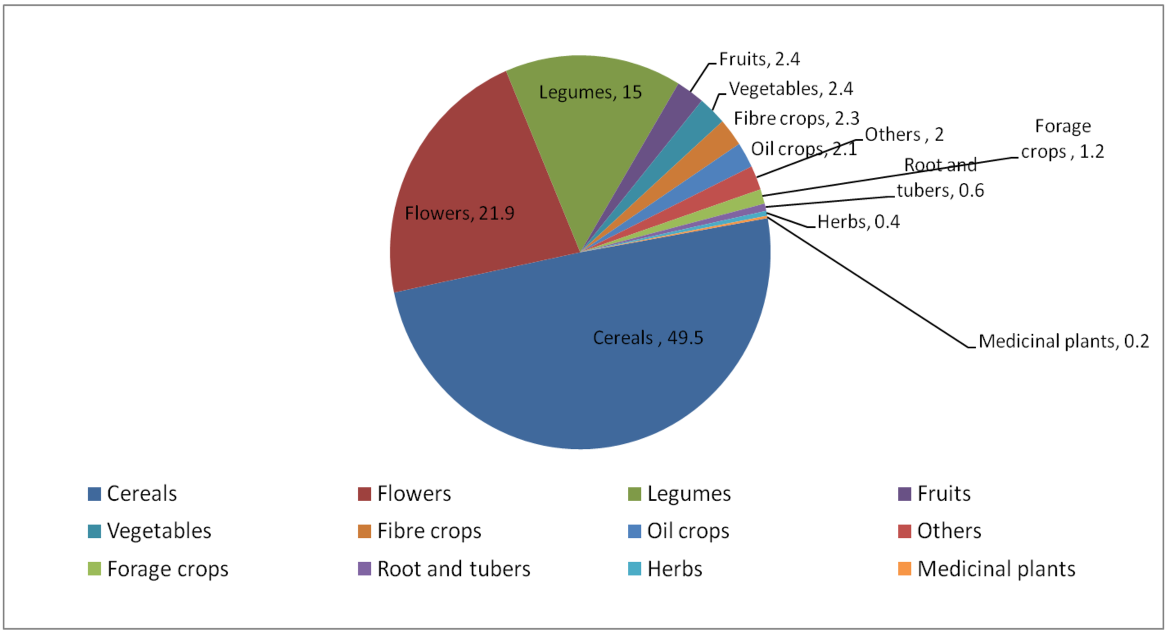

Fig 1: Mutant varieties in different crop plants http://www- aweb.iaea.org/nafa/pbg/mutation-breeding.html

Table 1: Varieties developed through Mutation breeding in MAP

Crop	Variety	Character
A. Medicinal crops		
Curcuma longa	BSR1	• Rhizomes contain higher curcumin content (4.2%)
	BSR2	• The crop yields 32 t/ha in a shorter crop duration of 240-250 days and has a curcumin content of 3.75%
	CO.1	Bold and orange yellow rhizomes, suitable for drought prone areas,
Catharanthus roseus	Dhawal	Leaf yield 2.0 t/ha and total leaf alkaloids content : 1.3-1.7%
Coleus forskohlii	Suphala	High yielding (15.93t/ha) year round cultivable variety
Solanum viarum	Arka Sanjeevani	Spineless variety
Plantago ovata	Niharika	Seed yield 10-11q/ha
	Mayuri	Seed yield 11q/ha
	Nimisha	Seed yield 10q/ha
Phyllanthus niruri	CIM-Jeevan	Phyllanthin content : 0.70-0.77% Hyphophyllanthin content : 0.32-0.37%
Hyoscyamus niger	Aela	Herb yield 37 —40 q/ha and total alkaloid content 0.32
Rauvolfia serpentina	CIM -Sheel	Dry root yield 25-30 q/ha and Reserpine content 0.030-0.035% in dry root
B. Aromatic crops		
Chamomilla recutita	CIM - Sammohak	Dry flower yield 7.53 q/ha and Oil yield 6.63 Kg/ha
	Vallary	Dry flower yield 7.0 q/ha and Oil yield 6.0 Kg/ha
Cymbopogon winterianus	CIM Jeeva	Herb yield 215 q/ha and Oil yield 285 kg/ha
	RRL-JOR-3-1970	39% more oil
Cymbopogan flexuosus	RRL-38, RRL-57, RRL-59, RRL(B)-14	68-100% more oil
	LM 81	Oil is colourless and out of the two isomers of citral, only citral-a is exclusively present. citral-b is negligible.
Mentha citrata	Kiran	Herb yield 508 q/ha and oil content 0.5%
Mentha piperita	Pranjal	Oil yield 90 kg/ha
	Tushar	Oil quality Menthol (33.3%), menthone (27.3%)
	Kukrail	Oil yield 90 kg/ha and oil content 0.5%
Mentha spicata	Neera	Herb yield 80 q/ha and Oil content 0.40%
Zingiber officinale	Suravi	Plumpy rhizome, dark skinned yellow fleshed,
Coriandrum sativum	RCr 684	Resistant to stemgall and less susceptible the powdery mildew.
Foenicum vulgare	RMt 303	Less susceptible to powdery mildew
	RMt305	Resistant to powdery mildew and rootknot nematodes
Cuminum cyaminum	RZ-223	Resistant to wilt, superior in yield and seed quality over RZ-19

Future Prospects

Mutation in association with the new technology of genetic engineering will constitute tools of plant breeders in near future. Although most of the varieties released so far has been developed from a mutation in combination with the direct selection. In the present era *in vitro* culture and molecular methods have resulted in the creation of new and wide paradigm in the utilisation of mutation breeding for crop improvement. Recently, heavy ion beam irradiation has emerged as an effective and efficient way of inducing mutation in many plant varieties because of its broad spectrum and high frequency (Hayashi *et al.*, 2007) [26]. The impact of induced mutation crop improvement resulted in release of 3248 mutant varieties officially registered in Food and Agriculture organization (Kharkwal, 2018) [27]. In *in vitro* culture techniques, a small amount of tissues and calli can be subjected to mutagenesis for the betterment of crop species (Xu *et al.*, 2012) [28].

In future development of *in vitro* cell selection techniques for disease resistance would be equally important. The induced mutation has also proved useful in the preparation of genetic maps that will facilitate molecular marker assisted plant breeding in future (Schwarzacher., 1994) [29]. The direct use of mutation in the development of molecular maps in structural and functional genomics could lead to rapid improvement of plant yield and quality.

Conclusion

Genetic variability in medicinal and aromatic crops is very narrow, crop improvement using conventional breeding approaches for high yield of biomass and increase in the active principles takes a long period. Hence, induced mutagenesis is one of the most important approaches for broadening the genetic variation and diversity in crops to circumvent the bottleneck conditions. Though induced mutagenesis is a decades old technique, demonstrably can contribute to unleashing the potentials of plant genetic resources and thereby avail plant breeders the raw materials required to generate the envisaged smart crop varieties. Crop varieties generated through the exploitations of mutation breeding are significantly contributing towards long sustainable yield.

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