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REVIEW

Induced mutagenesis for genetic improvement of *Allium* genetic resources: a comprehensive review

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Abstract The genus *Allium* is one of the largest monocotyledon genera having 1000 designated species with commercial and economic significance. *Allium cepa* L. (onion) and *Allium sativum* L. (garlic) are the most imperative edible species of this genus which are cultivated and consumed globally. Despite that, not much systematic and focused research has been carried out in these crops due to constraints like biennial nature, high crossability, cross or sexual incompatibilities/limited sexual reproduction, obligate apomict, large genome size, high heterozygosity, etc. To create genetic variability, induced mutagenesis is the optimum and best alternative for *Alliums* especially for the development of genetically improved and wider adaptive cultivars under changing climatic scenario. Development of new cultivars having tolerance against various biotic and abiotic stresses needs continuous efforts and attention of the breeder. For that, sufficient and wide genetic variation in the germplasm is the driving force for the breeders to select best genotypes. Development of mutants can be an alternative breeding strategy since mutations cause heritable genetic variations, which provide the eventual foundation for the evolution of new cultivars, forms or species. Such variations could be created artificially through various chemicals or physical agents, known as mutagens. Mutation breeding is an efficient and conventional method of crop improvement. With the aid of modern omics and molecular markers, allium breeding could be accelerated to develop desired products under rapidly climate scenario. This is the first comprehensive and detailed review on induced mutagenesis and mutation breeding in alliums.

Keywords *Allium* · Mutation breeding · Genetic resources · EMS · Gamma radiations · IAEA

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Introduction

The genus *Allium* belongs to the family *Amaryllidaceae* which is considered to be the biggest genus of the petaloid monocotyledons comprising 1000 described species (Khassanov 2018). This genus is of an epitomized commercial importance since it contains economically important vegetable and ornamental crop species such as *Allium cepa* L. (onion), *A. sativum* L. (garlic), *A. ampeloprasum* L. (great headed garlic), *A. schoenoprasum* L. and *Allium cepa* var. viviparum (Metzg.) Alef. etc. (Fritsch and Friesen 2002). Onion, garlic, leek, Japanese bunching onion and shallot are considered to be the edible Allium crops. Besides these, there are many other species of this genus which have been consumed by humans across the world (van der Meer 1997). *Allium aflatunense* B. Fedtsch. species has been used for ornamental purposes. The natural and artificial selection has resulted in the establishment of various plant types in domesticated species.

Onion and garlic are the most important commercial allium crops which are cultivated across the world (Figs. 1 and 2). Globally, demand for alliums has increased significantly than before due to its diverse use in cooked, processed and raw food as well as its medicinal value. But, changing climatic conditions and burgeoning human population are posing the prime challenge for sustainable production of bulbous crops worldwide. The sustained supply of high quality and high-yielding onion and garlic cultivars is the main consideration under various biotic and abiotic stresses occurring due to the swiftly altering environmental situation. Development of high yielding cultivars with improved bulb quality and tolerant to various abiotic and biotic stresses is a very challenging task in allium crops due to intensive cross pollination nature, high inbreeding depression, biennial crop habit, big genome size, etc. Efforts are being attempted to utilize wild species such as *A. fistulosum* L., *A. roylei* Stearn etc. but the success rate is very low. To improve commercial cultivars of onion and garlic in a shortened period, mutation breeding is one of the lucrative options for crop improvement and also for a specific trait breeding program. Induction of mutations has been exploited in various field and horticultural crops (Ahloowalia and Maluszynski 2001; Ahloowalia et al. 2004; Ali et al. 2012). Under erratic and unpredictable climatic conditions, there should be a basic requirement to develop new cultivars regularly, having a broad genetic base plant population to cater to the human population in future and for sustainable production of allium crops.

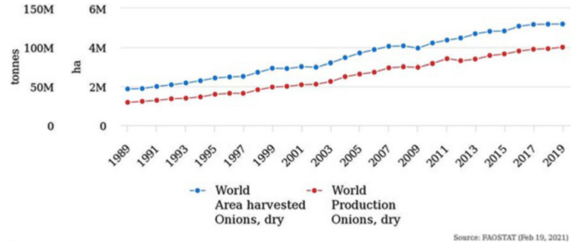

Fig. 1 Global dry onion production (million tons) and harvested area from 1989 to 2019 (FAOSTAT 2021)

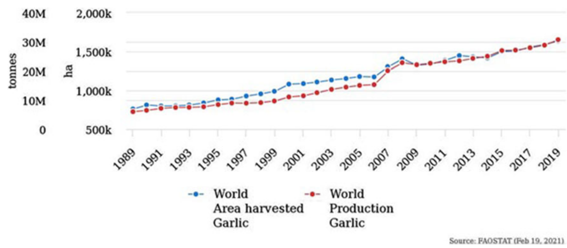

Fig. 2 Global garlic production (million tons) and harvested area from 1989 to 2019 (FAOSTAT 2021)

Plant genetic improvement is a continuous and constant phenomenon for bridging the gap between demand and supply of food by the burgeoning population worldwide. Since the ancient periods, man has been honing skills for various crop improvement approaches. The base of all such approaches is to exploit natural genetic variability present in the particular plant species. With time, few biologists began working on the scientific and systematic crop improvement methods. After the revolutionary research on creation of artificial variability by Stadler (1928) in barley, it was comprehended that there is no need to be dependent only on the existing genetic variation. Since then, crop improvement through mutation breeding has been an imperative and significant tool for the plant breeders.

Mutations can be generated through natural means or artificially through mutagens. Mutations are the heritable sudden changes in the DNA or genetic material of the individual and the same has been reflected in the form of phenotypes. Induced mutagenesis gives variability but results are always unpredictable (Lawande et al. 2009). Genetic heritable variations created by mutagen are very efficient since spontaneous mutation frequency is very low. All types of mutations create diverse and base genetic material which is very important for plant breeders for genetic crop improvement and development of widely adopted cultivars (Wattoo et al. 2012). It is well documented that mutagenesis induction facilitates the development of improved cultivars at a higher rate (Ahirwar and Verma 2016).

Artificial induced mutations are mainly categorized into chemical and physical mutagenesis depending upon the nature of mutagenic agent. As per available literature, mutagenesis studies in onion and garlic are scanty as compared to other field and vegetable crops. Under induced mutagenesis for genetic improvement of various alliums, some researchers conducted experiments to test efficiency of various chemical and physical mutagens (Kaul 1985; Kirtane et al. 2000; Hassan and Ahmad 2000; Amjad and Anjum 2002; Kirtane and Dhumal 2004; Hao and Cheng-Zuo 2006). Most of these studies used radiations and a perusal of literature clearly indicates that there is a need for chemical mutagen sensitivity investigations in onion. Various chemical and physical mutagenic agents are exploited in Alliums but more preference has been given to ethyl methane sulfonate (EMS) and gamma rays (Tables 2 and 3). Through mutation breeding, only eight mutant varieties have been released and registered at FAO site (Table 1) as per IAEA (2020). Very less genetic resources of alliums mutant lines have been generated worldwide by using induced mutagenesis. The conventional mutagenesis approaches create random and accidental mutations across the genome that lead to changes in non-target traits and are always unpredictable. With the onset of advanced mutagenesis and genome editing approaches such as CRISPR/Cas9, scientists alter only target gene(s). Till date, no report has been available regarding genome editing in Alliums. Unfortunately, whole genome sequence data is also not available till date.

In this review, we are trying to compile mutation induction approaches and their utilization in the genetic improvement of alliums. Different methods to screen the mutagenized population is depicted in Fig. 3 as per Sikora et al. (2011). The current review would be itself the first one specific to alliums as per literature available.

Challenges in garlic breeding

Garlic is an asexually propagated crop, so conventional breeding is very difficult and problematic to generate genetic variations in commercial cultivars. It can be said that creation of genetic variation through conventional breeding approaches is scanty or even impossible due to the absence of sexuality. Therefore, breeders have only few options such as clonal selection, induction of mutations using chemical or physical mutagens, somaclonal variations, genetic transformation or genome editing to create variations at genetic level for the development of improved cultivars (Kamenetsky 2007; Robledo-Paz and Tovar-Soto 2012). Besides this, the genome size of garlic is huge and negligible genomic resources have been available. Being an important crop, not much significant research progress has been observed in garlic.

Induced mutagenesis

For induced mutagenesis, basic steps consist of (1) mutation induction, (2) mutation detection, and (3) mutant line development. Induction of mutations is quick, might take seconds, minutes, or some hours, while its detection is critically a long process depending upon the species and trait of interest but with the help of modern omic tools such as high-throughput and other molecular methods. However, isolating mutant line development is again laborious as is in the case of traditional breeding methodology but can be achieved faster through marker-assisted breeding (MAS), TILLING, next-generation sequencing (NGS) and in vitro culturing techniques (Joung and Sander 2013; Zheng et al. 2013; Bado et al. 2015).

Two types of mutagens i.e., chemical (EMS, sodium azide etc.) and physical irradiation (Gamma and X-rays) have been extensively utilized for induced mutagenesis in field and horticultural crops globally. Chemical mutagen i.e., EMS is widely used for induced mutations in plants since it causes point mutations that are most desired and suitable for creating nonsense and missense mutations. Irradiations usually cause deletion and rearrangement of chromosomes. The foremost step for induced mutagenesis is the estimation of the most appropriate dose application to the particular crop species or genotype. In this section, we have discussed types of mutagens and crops separately.

Chemical mutagenesis

To create genetic variations in various plant species, use of chemical mutagens is considered to be the most convenient and effective method. Among alliums, EMS, EI, DES, SA etc. have been explored for chemical mutagenesis studies (Kataria and Singh 1989a, b, c; Choudhary and Dnyanansagar 1982; Hassan and Ahmad 2000; Joshi et al. 2011; Hassan et al. 2014; Ahirwar and Verma 2016; Banjare et al. 2017; Kirtane 2018). From the available literature, it can be inferred that EMS is the most efficient and widely used chemical mutagen in plants for inducing mutations due to its high efficiency and potency and

Table 1 Mutant cultivars of genus Allium approved by IAEA around the globe

SN	Variety Name	Country of release	Mutagen	Mutagen dose	Seed/Part	Parent genotype	Use of mutant	Improved trait	Releasing Year	ID No
Onion (*Allium cepa* L.)										
1*	Compas	Netherlands	X-rays	150 Gy	Seed	Grobol	Direct	Very firm, long-self-life, for mechanical handling and uniformity	1970*	
2*	Brunette	Netherlands	X-rays	150 Gy	Seed	Grobol	Direct	Earliness, high yield and good quality	1973*	
3*	KIK-11	Russia	N-nitroso-N-ethyl urea (NEU)	0.05%	Seed	Caratalinskii x Valensia cross and Octjabriiskii	Cross of two mutants (Caratalinskii x Valensia) x mutant from Octjabriiskii	High yield and suitable to universal using	1991*	
4*	Tabys (KIK-13)	Russia	NEU	0.05%	Seed	October	–	Higher yield	1993*	
5	KEWEI HUANG No.4	China		With 5 doses He–Ne laser			–	High quality and high yield		
6*	Binapiaz-1	Bangladesh	Gamma rays	75 Gy	Seed	BARI Piaz-2	–	Higher seed yield in winter season, and Fresh and dry bulb yield in summer season Longer shelf live	2018*	
7*	Binapiaz-2	Bangladesh	Gamma rays	100 Gy	Seed	BARI Piaz-2	–	More seed in winter season Higher seed yield Longer shelf life	2018*	
Chinese garlic (*Allium macrostemon* Bunge)										
8*	Ningsuan 1	China	Gamma rays	15 Gy		Landrace	–	High yield, good quality, drought tolerance, diseases resistance, good storage and transportation	1990*	

*Registered at https://mvd.iaea.org/

easy to use to create stable point mutation such as missense, nonsense and silent mutations in the whole genome (McCallum et al. 2000a, b; Kostov et al. 2007; Wattoo et al. 2012). Furthermore, it has also been seen that EMS mutagen shows more efficiency and effectiveness than physical mutagens for plants in the form of a variety of phenotypes (Dhanayanth and Reddy 2000; Bhat et al. 2005). The details of chemical mutagenesis in alliums have been summarized in Table 2.

Onion In onion, EMS is mostly preferred chemical mutagen to create genetic and morphological variation to improve particular trait of interest such as quality improvement, disease resistance, male sterility etc. Chemical mutagens are found to be more effective compared to physical mutagens (Kharkwal 1998; Kirtane 2018). However, efficiency of chemical mutagen (EMS) would be higher if used in lower concentration for plants (Joshi et al. 2011; Wattoo et al. 2012). Induced mutagenesis studies in onion were initiated during the 1970's. Earlier reports using chemical mutagens in onion (Banerjee and Sharma 1971; Bhamburkar and Bhalla 1980; Kataria and Singh 1989a, b, c, 1990) revealed the importance of induced mutagenesis but not much systematic and sustained work was attempted.

Kataria and Singh (1989a) conducted mutagenic experiment by taking three chemicals such as EMS (0.05–0.2%), N-methyl-N-nitrosourea (NMU, 0.01–0.1%) and ethylene imine (0.05–0.2% EI) and one physical i.e., gamma radiation (7.5–15.0 kR) mutagens in three onion varieties Pusa Ratnar, Pusa Red and White Warangal. They reported that enhancing mutagen dose led to decrease in seed germination, survival, seedling height, bulb weight and seed setting. Among the genotypes, White Warangal was found to be the most sensitive to mutagen, displaying significant reduction in seed germination and survival. Furthermore, the same research group in another study assessed the frequency of chlorophyll mutants in M_2 population. They observed that gamma rays were two–threefold more effective than the chemical mutagens and 10.0 and 12.5 kR produced the highest mutation incidence. The order of varietal sensibility towards mutagen (mutagen effectiveness) was White Warangal > Pusa Ratnar > Pusa Red (Kataria and Singh 1989b). However, decline in shoot and root length was observed more noticeable with SA

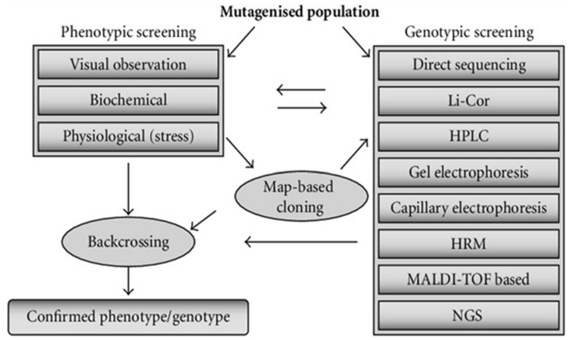

Fig. 3 Overview of different methods to screen a mutagenized population and to develop a new stable character. Adapted from Sikora et al. (2011)

treatment than EMS mutagen. On the hand, it was interestingly observed that seed germination activity was influenced more than growth of seedlings with chemical mutagen treatment (Joshi et al. 2011).

Enhancing mutagen dose caused adverse effects on biological activities of plants which might be due to inactivation of cells because of mitotic disturbances or abnormalities in chromosomes at higher mutagen dose, eventually leading to declined overall plant growth (Zargar et al. 1994; Kirtane 2018). Recently, Indian scientist conducted comparative study to determine mutagenic effectiveness and efficiency of physical gamma radiation (2- 12kR) and chemical mutagens sodium azide (0.1–0.7% for 4 h) alone and in combination in onion cultivar N-241 in terms of pollen sterility, lethality and chlorophyll mutants. The data obtained from the experiment elicited that sodium azide alone was most effective. However, lethality-based efficiency was found to be high with gamma radiation, but combined treatments gave high efficiency in pollen sterility compared to only physical or chemical mutagen treatment (Kirtane 2018).

Hassan and Ahmad (2000) studied the effect of chemical mutagens on diploid (*Allium cepa)* and tetraploid (*Allium vineale* L.) allium species for the induction of mitotic chromosomal abnormalities. It was found that all mutagens were effective to cause chromosomal aberrations in both species, but tetraploid species revealed more mitotic anomalies compared with the diploid species. Furthermore, it was found that all chemical mutagens caused aberrations in chromosomes but chromosomal irregularities were augmented due to EMS (0.05–0.20% concentration range of EMS being used in experiment) with contrast

Table 2 Mutation studies in Allium species using chemical mutagens

SN	Country	Mutagen	Dose (s)	Duration	Genotype(s)	Treatment given to	Purpose/Objective	References
Onion (*A. cepa* L.)								
1	Bangladesh	EMS	0.05, 0.10, 0.15 and 0.20%	24 h	*A cepa* (Diploid) & *A. vineale* (tetraploid)	Seed	Induction of mitotic chromosomal aberrations	Hassan and Ahmad (2000)
		DES	0.02, 0.06, 0.10 and 0.15%					
		EI	0.0025, 0.005, 0.0075 and 0.01%					
2	India	EMS			Pusa Red, Pusa Ratnar and White Waranagal	Seed		Kataria and Singh (1989a, b, c)
		NMU						
		EI						
3	India	EMS	0.2%	6 h	*A. Cepa*	Bulbs	Meiotic studies. Translocation/ inversion induction heterozygote	Ahirwar and Verma (2016)
				12 h				
				18 h				
				24 h				
4	India	EMS	0.1, 0.15, 0.2, 0.25%	4 h	B-780 L-28, N-241, Bima Raj, Bhima Red and Phule Samarth	Seed	To test the efficacy of chemical mutagens	Joshi et al. (2011)
		SA	0.1, 0.3, 0.5, 0.7%					
5	India	SA	0.1%, 0.3%, 0.5% and 0.7%	4 h	N-241	Seed	Mutagenic Effectiveness and Efficiency	Kirtane (2018)
6	India	EMS			White Desi, Nasik Red and Bellari Red	Seed	Differential mutagenic sensitivity	Bhamburkar and Bhalla 1980
		Mitomycin						
Garlic (*A. sativum* L.)								
1	India	EMS	0.1%, 0.4%, 0.8%, 1.2% and 1.6%	10 days	IG-2010–3-2, IG-2009–11-1 and Agrifound White	Cloves	Sprouting and survival characteristics	Banjare et al. (2017)

Table 2 continued

SN	Country	Mutagen	Dose (s)	Duration	Genotype(s)	Treatment given to	Purpose/Objective	References
2	China	EMS	0.2, 0.4 and 0.5%	1, 2 and 3 h	Yong Nian Daxuan and Zhong Mou	Callus	In vitro performance of callus under EMS	Hassan et al. (2014)
3	India	EMS	0.05 to 0.55%	6 and 12 h	G-41	Half cut cloves	To calculate the LD_{50}	Mahajan et al. 2015
		SA	0.01 to 0.40%					
		Colchicine	0.05 to 0.55%					
4	India	EMS	100, 200 and 300 mM	2, 4 and 8 h	Landrace	Cloves	Induced mutagenesis	Kaul (1985)
		DES	20, 25 and 30 mM	1, 2 and 3 h				
Pran (*A. cepa* var. *viviparum*)								
1	India	EMS	100, 200 and 300 mM	2, 4 and 8 h	Local Landrace from Kashmir	Bulbs	Induced mutagenesis	Kaul (1985)
		DES	20, 25 and 30 mM	1, 2 and 3 h				

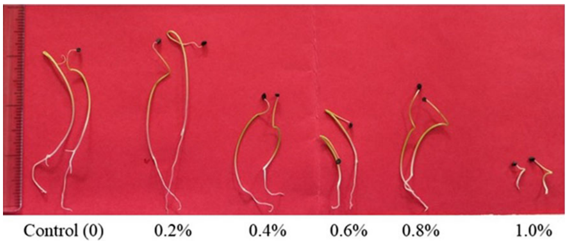

Fig. 4 Response of onion variety Bhima Dark Red to varying EMS concentrations

to other chemical mutagens. In another experiment, two chemical mutagens (Sodium Azide SA and EMS) were used to test efficacy for six Indian onion cultivars namely N-2-4-1, B-780, L-28, Phule Samarth, Bhima Red and Bhima Raj. Seeds of all genotypes were treated with EMS (0.1, 0.15, 0.2, 0.25%) and SA (0.1, 0.3, 0.5, 0.7%) for 4 h. Seed germination and seedling growth were analyzed and significant differences ($p < 0.01$) were observed in genotypes and treatments. Except for two varieties i.e., Phule Samarth and Bhima Red, lowest dose (0.1%) of both mutagens EMS and SA elicited stimulatory effect on seed germination. While at the same concentration (0.1%), shoot length was increased in L-28 and Bhima Red by SA and in Phule Samarth by EMS compared to untreated seedlings. In all the cultivars, higher doses of both mutagens caused significant reduction in germination, shoot and root length. However, this reduction has been observed more in case of SA than EMS treatment. This study confirmed that lower doses of chemical mutagens could be appropriate to induce genetic variation in onion (Joshi et al. 2011). Similarly, it has been documented that chemical mutagen sensitivity is genotype specific, depends on nuclear (chromosome number & size, number and position of centromere, polyploidy level, content of nuclear DNA) and extranuclear (cytoplasmic factors, seed weight and seed coat moisture content and seed weight) factors (Datta 1984, 2002; Wani and Khan 2006; Khan and Goyal 2009; Datta et al. 2011), for induced mutagenesis in plants. However, higher mutagen dose causes undesirable and adverse effects in terms of genetic impairment and poor plant/seedling growth (Joshi et al. 2011).

In Indian onion population, fifty needle punched healthy onion bulbs were treated with 0.2% EMS for 6, 12, 18 and 24 h. Treated bulbs were planted in earthen pots and appropriate size young flower buds were taken for meiotic studies. Anthers and pollen mother cells (PMCs) were examined with 2% iron acetocarmine for suitable stages of meiosis. Two onion mutants were identified from treated bulbs grown from

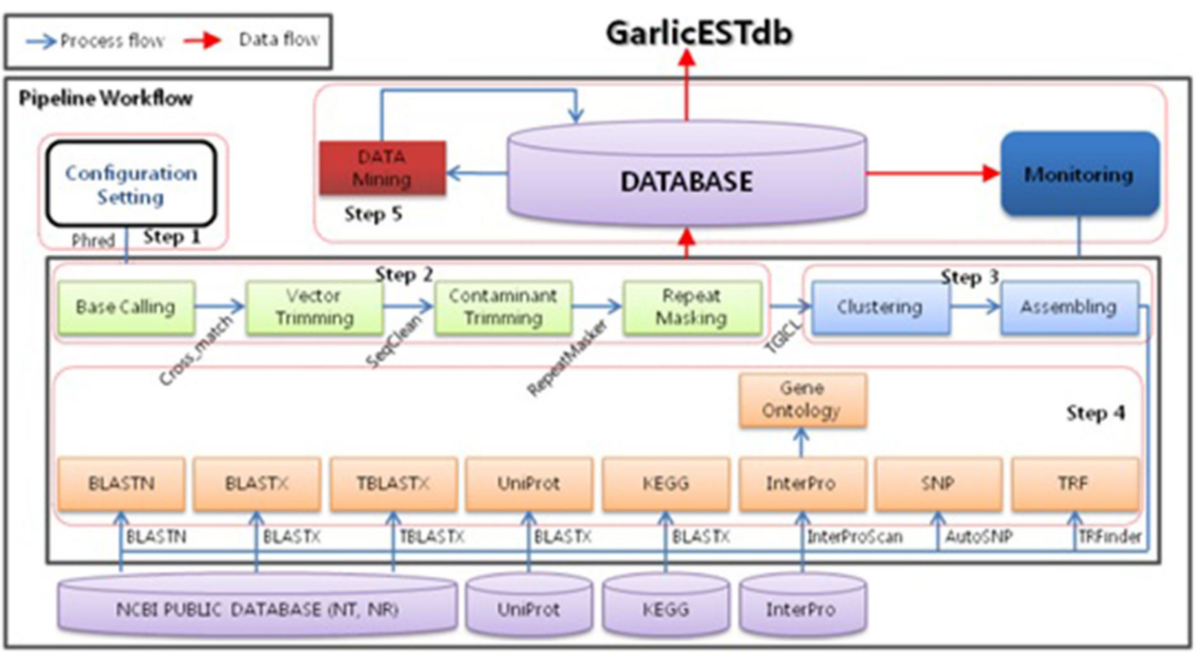

Fig. 5 Garlic EST database (GarlicESTdb). Adapted from Kim et al. (2009)

0.2% EMS for 12 h (translocation heterozygote) and 18 h (inversion heterozygote). The translocation heterozygote exhibited the formation of a chain of four chromosomes in majority of PMCs at diakinesis/metaphase-I. At anaphase-I, the translocation heterozygote displayed various abnormalities such as laggards, unequal distribution, micronuclei, etc. Whereas, inversion heterozygote mutants exhibited many chromosome arrangements at anaphase/telophase-I and II during meiosis. Furthermore, it was also noticed that fertility of pollen remains very less in both mutants (Ahirwar and Verma 2016).

The significant decline in seed germination after mutagen treatment might be due to inhibition or interruption of some biological and physiological activities essential for initiation of seed emergence comprising enzyme activity, hormonal imbalance and mitotic inhibition (Khan and Al-Qurainy 2009; Joshi et al. 2011; Kulkarni 2011). Correspondingly, auxin destruction, deviation in ascorbic acid content, physiological and biometrical instabilities, mitotic division suppression, unbalanced chromosome distribution and fragmentation contributed to reduction in shoot and root growth upon mutagen treatment (Singh 1974; Ussuf and Nair 1974; Datta et al. 1992; Joshi et al. 2011). Singh et al. (2021) reported that 0.6 per cent EMS is the optimum dose for short day onion cultivar Bhima Dark Red and can be used for development of onion mutants (Figs. 4 and 5).

Garlic Induced mutagenesis through chemical mutagen has been extensively explored for genetic improvement of various horticultural crops, where any variations would be of economic value. However, induced mutations could be a potential tool for garlic improvement being asexually propagated crop, but very inadequate and systematic research have been attempted. In the preliminary and initial studies, cloves of garlic were exposed with three chemical mutagens namely EMS, dES and ethyleneimine (EI) and one physical mutagen of gamma rays to create genetic variations. It was observed that sprouting percentage, survival and height of sprout declined with increase of dose or mutagen concentration. Furthermore, clove number and bulb weight improved with low concentration or dose of both physical or chemical mutagen (Choudhary and Dnyansagar 1980a, b).

Chemical mutagens have gained popularity since they are easy to use, do not require any specialized equipment, and can provide a very high mutation frequency. Of the chemical mutagens, EMS is today the most widely used. EMS selectively alkylates guanine bases causing the DNA-polymerase to favor placing a thymine residue over a cytosine residue opposite to the O-6-ethyl guanine during DNA replication, which results in a random point mutation. A majority of the changes (70–99%) in EMS mutated populations are GC to AT base pair transitions (Till et al. 2004, 2007; Sikora et al. 2011).

Selvaraj et al. (2011) studied the effect of ethyl methane sulphonate (EMS) and their combination treatments to improve bulb yield in garlic varieties'Mettupalayam' and'Ooty-1'. Based on radiosensitivity studies, two doses of gamma rays (2.5 and 5.0 Gy), four concentrations of EMS (15, 20, 25 and 30 mM for 8 h at temperature 25 ± 2 °C) and four combined treatments (2.5 Gy + 20 mM, 2.5 Gy + 25 mM, 5.0 Gy + 20 mM and 5.0 Gy + 25 mM) were employed. The magnitude of the positive or negative shift of the vegetative traits (number, length and breadth of leaves and diameter of pseudo stem) was greater after EMS treatments in both varieties. Genetic parameters, association analysis and regression analysis revealed the strong association of the bulb characters (diameter and volume) and clove characters (volume, weight and diameter) on bulb yield. For most of the traits stronger regression was exhibited by 2.5 Gy + 20 mM combination treatments.

In Raipur India, Banjare et al. (2017) investigated the effect of EMS on sprouting (%), survival (%) and lethal dose (LD50) of three garlic genotypes IG-2010-3-2, Agrifound White and IG-2009-11-1 in M_1 generation. Cloves were treated with 0.1, 0.4, 0.8, 1.2 and 1.6% EMS for 10 days. The results displayed that mutagenic sensibility varied with genotype in terms of sprouting (%), survival (%) and lethal dose (LD50). These biological parameters declined with enhancing mutagen concentration. The lethal dose of mutagen based on 50% reduction of sprouting and survival (%) also varied among the genotypes. Percent survival decreased significantly with higher dose.

Other alliums An obligate apomictic allium, *Allium cepa* var. *viviparum*, locally known as Pran, is intensively grown and popular in Kashmir valley, India (Singh et al. 1967; Koul and Gohil 1971; Kaul 1985). The induced mutagenesis was carried out in this *Allium* sp. through chemical mutagens viz., EMS (100, 200 and 300 mM for 2, 4 and 8 h) and dES (20, 25 and 30 mM for 1, 2 and 3 h) to determine LD50, create genetic variability and normal development of flowers (true botanical seed formation) by Kaul (1985) in Kashmir University, India. She indicated that mutagen concentration and treatment time is directly proportional to the decline in mitotic index and the chromosomal anomaly frequency. Not much significant morphological variations were observed in treated bulbs by both the mutagens. In another wild allium, tetraploid species (*Allium vineale*), locally known as wild garlic, was treated with three chemical mutagens (EMS, DES and EI) to study the chromosomal aberrations induced during mitosis. This species showed higher mitotic irregularities than *A. cepa*. Further, they observed that all three chemical mutagens were effective to induce chromosomal aberrations but EMS found the most effective (Hassan and Ahmad 2000).

Physical mutagenesis

Induction mutagenesis can broaden genetic diversity in plants (Van Harten 1998). Induced mutations serve as a complementary approach in genetic improvement of crops (Mahandjiev et al. 2001). For a long time, physical mutagens especially ionizing radiations have been extensively practiced for mutagenesis induction and several mutant cultivars had been developed through physical mutagenesis (Oladosu et al. 2016). Being a shorter wavelength, gamma radiation from radioactive cobalt (^{60}Co) is commonly used since this mutagen has more penetrating potential and could be used to irradiate delicate minute parts like pollens and complete plant or seedling (Oladosu et al. 2016). Nevertheless, gamma rays have been authenticated as being the most effective physical mutagen, specifically for creating large DNA fragment deletions (Kharkwal 2012). In comparison with chemical mutagenesis, physical mutagenesis especially gamma rays have been advantageous due to uniform penetrating ability in the treated tissue with high accuracy and reproducibility (Yamaguchi et al. 2003). In alliums, gamma rays have been used extensively for creating mutations especially in garlic and onion (Kaul 1985; Kirtane et al. 2000; Hassan and Ahmad 2000; Amjad and Anjum 2002; Kirtane and Dhumal 2004; Hao and Cheng-Zuo 2006; Kato et al. 2016).

Onion As per data provided by IAEA, Vienna, Austria, the first variety of onion 'Compas', developed by using physical mutagen, had been approved and registered by Netherland in 1970. For this, breeders treated seeds of 'Grobol' with X-rays @ 150 Gy and found the mutants having uniform bulbs, long keeping quality and suitable for mechanical handling (IAEA 2020). During the mid-seventies, Gaur and Nirale (1975) irradiated 20-day old whole onion plants with X-rays at 62.5, 125 and 250 R and found that the first two doses had no effect and elongation of leaves appeared to be radiosensitive. They summarized that growth of bulb is less receptive than growth of leaves. Gamma rays irradiated seeds of onion exhibited adverse effects on seed germination (%), seedling survival and height in three Indian onion varieties. In the same study, it was also observed that onion varieties exhibited varied radio-sensibility towards gamma rays (Bhamburkar and Bhalla 1980). Ahirwar (2015) treated onion seeds with 5, 10 15, 20 and 25 kr of gamma rays. Chromosomal abnormalities such as dicentric, fragment, tricentric, minute, deletion, bridge and micronuclei were observed during mitosis in root tip and such deviations were enhanced whereas seed germination declined with increasing radiation dose.

Apart from wide use of gamma radiation for induced mutagenesis in plants, but with very low dose of it, it also has potential to improve yield and quality in plant species (Watanabe et al. 2000; Amjad and Anjum 2007). Such quality of gamma radiation is highly specific to species and genotype even within species (Ahmad and Qureshi 1992; Kumar and Chaudhary 1996). Similarly, Amjad and Anjum (2007) irradiated onion seeds *cv*. Ailsa Craig with gamma rays with different doses to enhance ageing to investigate whether it augments or ameliorates the adverse effects of irradiation. Seeds of Ailsa Craig were treated with 0, 10, 20, 40, 80 and 100 krad gamma rays and subjected to accelerated ageing (RH 100% at 42 °C temperature) for 12 h. The significant effects of rays were observed for normal seedlings (%), seedling growth and abnormality types (%), whereas, no effect has been observed on final germination (%), seed viability, germination speed and time (50%) and conductivities of seed leachates.

Accelerated ageing after irradiation exhibited significant impact on seed parameters such as viability, final germination (%), conductivities of seed leachates and normal seedlings (%). No dose of radiation (10–100 krad) significantly influenced seed viability, so it is clear evidence that gamma radiation is not causing any physical injury to onion seed that will lead to seed death immediately. But accelerated ageing and gamma irradiated treatment interaction significantly affected seed viability. Furthermore, they revealed that all seed parameters were significantly affected by the interaction of accelerated ageing and dose of radiation. To repair damaging effects of gamma rays, accelerated ageing is helpful. So, it could be concluded that onion seeds should not be exposed to adverse environmental conditions after irradiation.

Kirtane (2014b) treated seeds of onion var. N-241 with sodium azide and gamma radiation alone and in combinations of various doses and concentrations. She observed four types of chlorophyll mutants such as *xantha*, *striata*, *albina* and *chlorina* induction in M_2 generation by different mutagens and combination of mutagens. However, a combination of two mutagen treatments exhibited higher effectiveness for the chlorophyll mutation induction among all treatments. On comparing inter-mutagens effectiveness, gamma rays were found to be more operative than sodium azide. The frequency of *albina* type among all mutants was reported the highest, but lowest found in striata type chlorophyll mutants.

Garlic In initial interesting studies of garlic, Choudhary and Dnyanansagar (1982) found that X-radiation was the most effective mutagen followed by EI, EMS and dES. It was also observed that various doses of X-rays declined mitotic activity and induced chromosome abnormalities in garlic (Gohil and Koul 1984).

In Philippine, Rosario and Miranda (1991) revealed that garlic is sensitive to gamma radiation at doses more than 0.8 Krad and beyond 1.0 Krad plants did not survive. They also found that radio-sensitivity response is genotype specific. In their study, cultivar 'Laguna Strain' displayed higher response to induced mutagenesis than llocos White. Taner et al. (2004) treated garlic cloves with 0, 5, 10, 15, 20, 25 and 30 Gy doses of gamma-rays and recommended 4.455 Gy as the effective dose. Shasghidhar et al. (2005) exposed cloves of garlic cv. Vannur Local with γ-radiation (0–9.0 Gy) and reported that percent survival declined with enhancing radiation dose. Furthermore, they reported that cloves treated with doses beyond 0.80 Gy were not able to survive. The radiation dose 0.40 Gy was determined the LD_{50} value for Vannur Local cultivar (Shashidhar et al. 2005). Most recently, El-Fiki and Adly (2020) noticed that sprouting of cloves was delayed with the enhancement of dose of rays (upto 20 Gy) and growth was arrested at 30 Gy.

Other alliums In Indonesia, locally grown Samosir shallot (*Allium ascalonicum* L.) is cultivated widely and is popular among people due to its typical pungent scent. Being biennial, Samosir shallot is a strictly cross pollinated and highly heterozygote with very low seed formation, so development of new cultivars and improvement through hybridization is cumbersome (Eady l995; Lawande et al. 2009). To broaden its genetic diversity for further genetic improvement, induced mutagenesis has been explored. Dried bulbs of this landrace were irradiated with gamma rays from 0 to 20 Gy to calculate LD50. Researchers found that enhancing radiation dose beyond 11 Gy significantly declined shoot growth compared to non-irradiated bulbs. They determined 11.60 Gy as LD50 value for this genotype using linear regression analysis (using Curve-fit Analysis software) on the basis of regenerated shoot percentage. Shorter plants having lower number of leaves have been observed in treated bulbs compared to non-treated ones (Sinuraya et al. 2015).

A perennial herb *Allium tuberosum* Rottler ex Sprengel is grown and popular in North East India and locally called *Maroi nakuppi*. This allium is propagated asexually through tuberous rhizhome. To create genetic variations, rhizomes of its locally popular variety '*Ningtham Sidabi*' were exposed to gamma radiations at 3–10 Gy dose. Average fresh and dry weight per tiller declined with the enhancing radiation. Also, it was noted that the lowest dose (3 Gy) of gamma rays stimulated the growth of plant in terms of plant height, average fresh and dry weight. This is the first induced mutagenesis study in this allium species. Henceforth, it would be useful for future breeding program (Devi 2020). A mutant developed from landrace through using 15 Gy gamma rays named 'Ningsuan-1' of Chinese garlic (*Allium macrostemon*), Bunge having drought tolerance, higher yield & quality and disease resistance (IAEA 2020).

In vitro mutagenesis

The frequency of natural mutations is low, so artificial induction of mutations is very common and effective way to develop new cultivars specifically in asexual propagated crops (Predieri and Divrgilio 2007; Humera and Javed 2012; Mustafa et al. 2015). In vitro culture has a great potential to improve effectiveness of mutation induction in various aspects such as (1) provides a wide choice of explant plant material for treatment like organs, tissues, in vitro axillary buds and cells; (2) less risk of obtaining chimeric plants; (3) more probability for mutated cells to express the mutation in the phenotype; (4) easy handling of large populations for mutagenic treatment, selection, and cloning of selected variants; (5) offers the possibility to rapidly execute the propagation cycles of subculture aimed to separate mutated from non-mutated sectors; (6) this technique also offers easy maintenance of phytosanitary conditions up to the final stage (Ahloowalia 1998). As garlic is the vegetatively propagated crop, this habit makes it challenging to create genetic variations through conventional cross breeding for the development of new cultivars. Hence, very limited scientific breeding approaches have been implied for the improvement of important traits in garlic (Agrawal et al. 2003). Breeding in garlic has been limited to clonal selection from land races or natural mutants. Combination of in vitro culture and mutagenesis in asexually propagated crops would be a great option to enhance genetic diversity and efficiency of this combination was established by Lee et al. (2003). The research on the use of mutagenesis in garlic breeding has not produced any significant results (Etoh and Simon 2002). There have been limited studies on the effect of mutagens on garlic, e.g. in vitro shoots (Peiwen et al. 1999) and garlic cloves (Al-Safadi et al. 2002). It was suggested that higher doses of gamma radiation (5–7 Gy) to in vitro conditions were evidently effective for mutant production (Mostafa et al. 2015).

Xixiang et al. (2014) conducted an experiment to determine the optimum concentration and duration of EMS on garlic callus in order to enhance the genetic variability of garlic. Garlic calluses were soaked in EMS with various concentrations (0.2, 0.4 and 0.5%) for diverse durations (1, 2 and 3 h). They reported that the reduction in the average number of sprouts per callus, total number of sprouts, number of sprouts forming plantlets, plantlet length and root numbers were observed with increase in the concentration of EMS and its duration of exposure. More specifically, the maximum value for the aforementioned parameters were recorded when callus was treated with lowest EMS concentration (0.2%) for a short duration (1 h). However, the maximum root length was observed in plantlets formed from callus was subjected to 0.2% of EMS for 2 h. Moreover, highest leaf numbers were obtained from 0.4% of EMS for 2 h. In contrast, the treated callus with 0.5% for 3 h gave the biggest bulblet diameter.

Mutagenesis by genome-editing approaches

With the invention of new and precise molecular tools and technologies, plant molecular biologists advanced their crop improvement program through manipulating the genome at desired loci. On the same line, genome editing of plant species is a recent and swiftly advanced molecular tool by which precisely targeted and desired mutations could be done in the genome. The recent cutting-edge technology of genome editing known as clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 eased the targeted mutagenesis in crop plants. The greatest advantages of this technology are that it does not come under transgenic modification and is much superior than induced mutagenesis through conventional methods. Through this tool, scientists are enabled to develop several gene knockout mutants which are quite beneficial for further crop improvement program. Besides few field and horticultural crops, exploitation of this technology is very limited. As per literature available, no report has been found with respect to alliums. In future, we could expect to explore such technologies to develop new commercial varieties of onion and garlic having specific trait under changing climate scenario to cater the burgeoning population of the globe.

Mutation for biotic and abiotic stresses

Mutation breeding has been used to improve garlic resistance to white rot disease (Perez-Moreno et al. 1991; Al-Safadi et al. 2000; Nabulsi et al. 2001).

White rot disease caused by fungus (*Sclerotium cepivorum*) is one of the serious challenges to sustainable garlic production worldwide (Schwartz and Mohan 1995). Due to non-availability of resistant genetic sources, induced mutagenesis has been exploited to develop resistance against this devastating disease (Perez-Moreno et al. 1991; Al-Safadi et al. 2000; Nabulsi et al. 2001).

In Syria, an induced mutagenesis experiment was conducted in two Garlic *cvs*. Kissvany and Yabroudy using gamma irradiations for fungal disease white rot (*Sclerotium cepivorum*) resistance and for storability improvement. Cloves were exposed to various doses of gamma rays (4, 5, 6, and 7 Gy), then planted in the field and advanced the generation up to M_4 to identify stable mutants. In the M3 and M4 generations, high selection pressure was applied by inoculating fungus sclerotes to the cloves and planting them in a soil previously planted with infected garlic plants. After harvesting, bulbs stored under natural conditions and then planted to advance generation. After the M4 generation, scientists isolated 24 mutant lines of both garlic cultivars having white rot disease resistance and high storability without any reduction in size of bulbs. Mutants obtained from cv. Kisswany exhibited about 3% disease compared to 29% in the original plant material, whereas mutants from Yabroudy showed less than 5% infectious percentage as compared to 20% in the control (Al-Safadi et al. 2000).

Mutation breeding for quality and nutritional status

The primary and foremost aim of every breeding program is to ensure food and nutritional security by developing high yielding resistant cultivars of a particular crop especially under changing climatic conditions. About 40 per cent of the global population is malnourished mainly relating to vitamin A, iron, and zinc because of insufficient food intake, less nutrient bioavailability and poor nutritional quality (Ramakrishnan 2002; Jain and Suprasanna 2011). To enhance quality and nutritional value in plants, induced mutagenesis could play a significant role and the same is evidenced by releasing 776 mutant varieties globally having high nutritional quality (Kharkwal and Shu 2010; Jain and Suprasanna 2011).

Kirtane (2014a) recorded that the combination of SA (3%) and gamma radiation (6 kR) resulted in higher chlorophyll and total sugar content whereas 8kR + 0.3% SA elicited maximum protein content in onion cultivar N-2-4-1. They concluded that combination of lower doses of chemical and physical mutagens were effective as stimulatory for biochemical parameters than higher doses, though high doses were inhibitory for onion. Park et al. (2004) reported that high doses of γ-radiation retarded the emergence of garlic sprouts and reduced the survival rate of the plants. SOD and POX activities increased with 5 and 10 Gy γ radiation treatment. Second generation of mutagenized garlic recorded normal SOD activity and reduced POD activity (46% of the control). DPPH radical 6 scavenging activity of garlic clove was significantly increased in the second generation of mutagenized garlic to IC50 mg compared to the control.

Onion bulbs were exposed to various doses of gamma rays to study the behavior of chromosomes and status of antioxidant enzymes on 3rd and 30th day respectively after irradiation to comprehend the radiosensitivity level. It was found that with enhancement of gamma rays dose, there was reduction in mitotic index and increase in chromosomal abnormalities, SOD-, GR-, APX- and Guaiacol peroxidase activity and MDA content. APX-, SOD-, GR- and Guaiacol peroxidase activity enhanced to its fullest after 3rd day of irradiation and thereafter reduced. Amplified positive significant variations of antioxidant enzymes with increase in dose of gamma ray were pre-requisite to guard alive cells from adverse influence of gamma radiations. Furthermore, it was also deduced that on 30th day the content of antioxidant enzymes was virtually equal or slightly improved in control and gamma ray treated bulbs. However, more chromosomal irregularities were observed due to stress induced by gamma rays on 3rd day. Thus, it could be established that there is a positive correlation between chromosomal aberrations and antioxidant enzymes related to the defense mechanism of living cell in onion (Datta et al. 2011).

In onion, pungency and tear inducing properties are of utmost pronounced quality parameters which are usually not preferred by the most of the consumers. People always prefer to choose low pungency onions, since it causes irritation during chopping of onions. So, day by day demand for low pungency onion is increasing. Low pungent onions have a biggest disadvantage of low storability due to high content of moisture relatively. To keep this in view, some scientists used heavy-ion radiations to generate mutants in onion for production of tearless onions which are not considered genetic modification or transgenics. Such mutant tearless onion cultivars would have more acceptance globally. Scientists from Central Research and Development Institute, Chiba, Japan attempted induced mutagenesis through neon-ion beam at 20 Gy in onion. They exposed seeds of Japanese onion cultivar 'Super-Kitamomiji' having specific traits such as high pungency, medium to large bulb size (180–260 g), long day type, day length sensitivity and high storage capacity. The bulbs obtained from M_1 seeds and next generation bulbs produced by selfing were assessed organoleptically for tear-inducing property or HPLC analysis of LF production. After subsequent repeating cycles of screening and seed production by selfing, two mutant bulbs, having tearless and non-pungent traits, from M_3 bulbs were isolated. About twenty bulbs produced from each identified bulb in M_4 generation and displayed no tear-inducing or pungency property after evaluating by sensory panelists. It was calculated that there was 7.5-fold less LF production level in mutant bulbs compared with the original normal onion and it was due to decline in alliinase activity, which was a result of low alliinase mRNA expression (< 1% in normal onion) and consequent low amounts of the alliinase protein (Kato et al. 2016).

El-Fiki and Adly (2020) studied that gamma irradiation causes adverse effect on 1,8-Dioxaspiro [4.5] decan-2-one 4- (2-aminothiazol-4-yl)-7,7-dimethyl-; MF: (C13H18N2O3S) compounds in the cultivar Balady. However, it declined by almost half and was not found in the Sids 40 cultivar nor its treatments. On the other hand, a positive effect was noticed on Diallyl disulfide; MF: (C6H10S2) and trisulfide, di-2-propenyl; MF: (C6H10S3) in both genotypes. This compound [2-Trimethylsilyl-1,3-dithiane]; MF: (C7H16S2Si) was not found in both cultivars but found only in Sids 40 cultivar with dose 10 Gy with value (5.51%). The compound of Methanone, [4-] [4-mthyl-5-(phenylmethyl)-4H-1,2,4-triazol-3-yl] thio]-3- nitrophenyl] 2-pyridinyl-; FM: (C22H17 N5O3S) was formed only with gamma radiation dose 10 Gy in both cultivars Balady and Sids 40 with a value of 1.75% and 2.65%, respectively, and was not formed with un-irradiated. Therefore, it can be concluded that the changes caused by physical mutagens like gamma radiation on the sulfur compounds in garlic exhibited enhancement, reducing, disappearing and formation of new compounds.

Registered *Allium* cultivars by IAEA, Austria

According to the International Atomic Energy Agency (IAEA) database, among 3319 cultivars, to date, six onion (*Allium cepa*) and one of Chinese garlic (*Allium macrostemon*) mutant varieties have been registered throughout the world using mutation breeding techniques (Table 1). The first mutant cultivar of onion namely 'Compas' was registered officially in 1970 from the Netherland. This mutant variety was developed through x-rays (150 Gy) physical mutagenesis and comprises improved traits like firmness, high storability, globe type with brownish/yellow skin, more uniformity, more dry outer leaves etc. In 2018, Bangladesh institute of Nuclear Agriculture (BINA), Bangladesh has registered two mutant onion cultivars i.e. Binapiaz-1 and Binapiaz-2. The released mutant cultivars possess particular characters such as higher yield, longer shelf life, early maturity, high quality etc. However, compared to other vegetable crops, very few mutant cultivars have been developed globally. Across the globe, various research groups are attempting mutagenesis approaches to develop cultivars having high yield, bulb uniformity, high quality, tolerance to various biotic and abiotic stresses under rapid changing and unpredictable climatic conditions. Some of them have achieved success in their attempt to modify or develop new traits in allium (Tables 2, 3 and 4).

Mutant/genomic resources of *Alliums*

The genus Allium is a huge and genetically diverse genus having more than 1250 species, including major cultivated crops such as garlic, onion, chives, leek and shallot (Kim et al. 2009). Currently, the interest in alliums is especially in garlic and highly in demand due to its nutritional and pharmaceutical properties including high blood pressure and cholesterol, atherosclerosis and cancer (Khar et al. 2020). There are no comprehensive databases available for Expressed Sequence Tags (ESTs) of garlic for gene discovery and future efforts of genome annotation. Up till 2009, no tool or database for garlic was available which could provide the information about annotations and ESTs. Later on, Kim et al. (2009) developed the GarlicESTdb using a pipeline system which offers

Table 3 Mutation studies in *Allium* species using physical mutagens

SN	Country	Mutagen	Dose(s)	Radiation Source	Genotype(s)	Treatment given to	Purpose/ Objective	References
Onion (*A. cepa* L.)								
1	India	Gamma	2, 4, 6, 8, 10 and 12 kR	^{60}Co	N-241	Seed	Mutagenic Effectiveness and Efficiency	Kirtane (2018)
2	Pakistan	Gamma	10, 20, 40, 80 and 100 krad	lICo	Ailsa Craig	Seed	Seed germination and viability, seedling growth	Amjad and Anjum (2002)
3	India	Gamma	20, 40 and 60 Gy	^{60}Co	*A. cepa*	Bulb	For chromosomal aberrations and enzyme related defense	Datta et al. (2011)
4	India	X-rays	62.5, 125 and 250 R	–	*A. cepa*	20-day old seedlings and other parts	Radio-sensitivity of X-rays on different plant parts	Guar and Nirale. (1975)
5	India	Gamma			White Desi, Nasik Red and Bellari Red	Seed	Differential mutagenic sensitivity	Bhamburkar and Bhalla (1980)
6	India	Gamma	5, 10, 15, 20 and 25kR	–	*A. cepa*	Seed	Chromosome aberrations and development of desired mutants	Ahirwar (2015)
7	Pakistan	Gamma	10, 20, 40, 80 and 100 krad for 5, 10, 20, 40 and 50 min, respectively	^{60}Co at the rate of 2 krad/ min	Ailsa Craig	Seed	Effect of post-irradiation ageing on seeds	Amjad and Anjum (2007)
8	Syria	Gamma	10 Gy for 7 days		Red and white cultivars	Seeds	Effect of gamma irradiation on yield	Al-Oudat (1989)
			1 Gy for 7 days			Bulblets and bulbs		
Garlic (*A. sativum* L.)								
1	India	Gamma	0.20, 0.40, 0.60. 0.60, 0.80, 1.00, 3.00, 5.00, 7.00 and 9.00 Gy		Vannur Local	Cloves	To find out LD-50	Shashidhar et al.. (2005)
2	Syria	Gamma	4, 5, 6, 7 Gy (Gy)	^{137}CS @ 8.7 Gy/ min	Kisswany and Yabroudt	Cloves	For white rot resistance and higher storability	Al-Safadi et al. (2000)
3	Turkey	Gamma	5, 10, 15, 20, 25 and 30 Gy	Cs137		Cloves	Effective Mutagen Dose (ED50)	Taner et al. (2004)

Table 3 continued

SN	Country	Mutagen	Dose(s)	Radiation Source	Genotype(s)	Treatment given to	Purpose/ Objective	References
4	China	Gamma	1, 3, 5 and 7 Gy	^{60}Co @ 0.7 Gy/ min	YongNian Daxuan and ZhongMou	Callus	To generate in vitro genetic variation	Mostafa et al. (2015)
5	Egypt	Gamma	10, 20, 30 and 40 Gy	^{137}Cs @ 1.3083 KGy/h	Balady and Sids 40	Clove	Morphological, molecular and organosulphur compounds characterization	El-Fiki and Adly (2020)
6	India	Gamma	3–21 Kr @ 3 Kr interval		G-41	Half cut cloves	To calculate the LD_{50}	Mahajan et al. (2015)
7	India	Gamma	0.1–0.5 Kr	Chamber 900 with ^{60}Co	Landrace from Kashmir Valley	Cloves	Induced mutagenesis	Kaul (1985)
8	Philippines	Gamma	0.5, 0.6, 0.8 and 1 Kr	–	llocos White and Laguna Strain	Cloves	For disease and pest resistance	Rosario and Miranda (1991)
Shallot (*Allium ascalonicum*)								
1	Indonesia	Gamma	1–20 Gy	Chamber A 4000 with ^{60}Co	Local Samosir shallot	Bulbs	Radio-sensitivity and plant growth	Sinuraya et al. (2015)
Pran (*A. cepa* var. *viviparum*)								
2	India	Gamma	0.1–0.5 Kr	Chamber 900 with ^{60}Co	Landrace	Bulbs	Induced mutagenesis	Kaul (1985)
Allium tuberosum Rottler ex Sprengel								
3	India	Gamma	3, 5, 7 and 10 Gy	^{60}Co	Ningtham sidabi	Rhizomes	Induced mutagenesis	Devi (2020)

access to all garlic EST resources and comprehensive database containing information about the cluster, annotation, protein domain, pathway, tandem repeat, single nucleotide polymorphism (SNP), etc. To carry forward this genomics research in garlic, polymorphism among garlic germplasm has been revealed through transcriptome sequencing in expressed regions which is helpful for diversity analysis and genetic map development (Kuhl et al. 2004; Martin et al. 2005; Gore et al. 2009; Duangjit et al. 2013; Ipek et al. 2015). Use of molecular markers like AFLPs, SSRs and SNPs have been identified (Ipek et al. 2005, 2015; Ma et al. 2009; Zewdie et al. 2005; Zhao et al. 2011). Until now, similar appearance and phenotypic plasticity of garlic varieties hinder their morphological classification. Molecular studies are challenging, due to the large and expected complex genome of this species, with asexual reproduction.

Table 4 Use of molecular markers to detect polymorphism among mutants in Allium

SN	Country	Marker	Primers	Polymorphic primer and sequence	Base genotype	Purpose	References
Garlic (*A. sativum*)							
1	Syria	RAPD	13	OPB-15 (GGAGGGTGTT)	Kisswany and Yabroudy	Resistance to white rot disease	Nabulsi et al. (2001)
2	Egypt	SCoT*	15	SCoT-4 (5'-CAACAATGGCTACCACCT-3') and 5 (5'-CAACAATGGCTACCACGA-3)	Balady and Sids 40	Organosulphur compounds characterization	El-Fiki and Adly (2020)

*Start Codon Targeted

Classical molecular markers, like isozymes, RAPD, SSR, or AFLP, are not convenient to generate germplasm core-collections for this species. The recent emergence of high-throughput genotyping by-sequencing (GBS) approaches, like DArTseq, allow to overcome such limitations to characterize and protect genetic diversity. Therefore, such technology was used in this work to: (1) assess genetic diversity and structure of a large garlic germplasm bank (417 accessions); (2) create a core collection; (3) relate genotype to agronomical features; and (4) describe a cost-effective method to manage genetic diversity in garlic germplasm banks.

Use of markers for mutant analysis

Along with conventional breeding approaches, molecular markers play a great role in advancing the crop breeding program. To detect polymorphism among generated mutants at molecular level in crop plants is to make it easy for the breeder to select tolerant or resistant plants at an early stage. Selection through morphological characteristics of mutant plants is very cumbersome and laborious, additionally not every mutant has an equal chance to be observed in a large population as compared to selection through molecular markers. In garlic, Random amplified polymorphic DNA (RAPD) markers were practiced to test eight mutants resistant to white rot disease (*Sclerotium cepivorum*) using 13 random primers. Among all, only one primer OPB-15 (GGAGGGTGTT) showed polymorphism during amplification (Nabulsi et al. 2001). In China, 16 novel microsatellites were recently used to detect mutations created through gamma radiated callus. The molecular analysis revealed genetic variation in the both genotypes (YN and ZM). Furthermore, it was observed that primer G-095 showed polymorphism in two seedlings regenerated from calli of genotype YN treated with 5 Gy radiation, while primer G-101 exhibited polymorphism in one seedling of ZM formed from calli treated with 7 Gy (Mostafa et al. 2015).

Most recently, El-Fiki and Adly (2020) designed fifteen SCoT primers to analyze variation in gamma irradiated two garlic cloves in Egypt. They got 43 (41.35%) and 31 (31.31%) polymorphic bands in Balady and Sids 40 cultivars, respectively. The average number of the amplified bands per primer was recorded varied in both genotypes i.e. 6.93 and 6.6. The polymorphic band number in cv. Balady was varied from 1 (20%) SCoT-36 primer to 6 (66.66%) SCoT-5 primer. While in Sids 40, the range was from zero (SCoT-16) to 6 (SCoT-5). In Balady, the average polymorphic information content (PIC) value and marker index (MI) are 0.29 (PIC < 0.5) and 0.12, respectively. On the other hand, in cv. Sids 40, the average value of PIC and MI were 3.63 (PIC < 0.5) and 0.105, respectively.

Challenges and constraints for mutagenesis induction in alliums

According to Bado et al. (2015), major limitations of mutagenesis induction in plants include random and unpredictable nature of mutagens. Hence a large population for getting trait of interest; before any selection, the population must be advanced minimum up to two generations; there is a large gap between genotype and phenotype; and finally, a wide gap in technology among developing and developed countries etc. Furthermore, nondestructive and cost-effective high throughput phenotyping and genotyping is very important to fasten the identification and screening of desired mutants (Schunk and Eberius 2012; Jankowicz-Cieslak et al. 2012; Bado et al. 2015).

Garlic (*Allium sativum* L.) has a long history of cultivation by asexual propagation due to obligate apomict. Due to its asexual nature, improvement of garlic has been limited as compared to onion (Khar et al. 2020). Mutation induction can increase the genetic diversity of plants (Van Harten 1998). Induced mutations serve as a complementary approach in genetic improvement of crops (Mahandjiev et al. 2001). Genetic improvement of onion by conventional breeding methods of hybridization is slow due to its high heterozygous, outcrossing and biennial nature. To augment this process induced mutagenesis could serve as a useful tool (Joshi et al. 2011).

Conclusion and future prospective

Use of induced mutagenesis to create genetic variations and broadening genetic base in various field and horticultural crop species in a shorter period have been known for a long period of time. Several milestone varieties in various crops have been developed through induced mutagenesis in developed and developing countries such as the USA, China, India etc. Apart from its great potential for genetic improvement of qualitative and quantitative traits, this technique has not yet been comprehensively explored for the development of improved and well adapted cultivars in various species of *Allium* especially garlic being a vegetatively propagated crop. Except onion and Chinese garlic, no commercial cultivar developed through mutagenesis in garlic has been registered. Various biotic and abiotic stress have become great challenges for alliums production, and with the changing climatic conditions new stresses are appearing. To counter such challenges, allium breeders need heritable genetic variation in the existing gene pool of desired traits. In asexually propagated crops like garlic and other alliums, mutation breeding would be the optimal method to create variation within a particular crop. Induced mutagenesis is a cost-effective and simple method for improving genetics and development of widely adapted and resistant cultivars (Ahloowalia et al. 2004; Mlcochova et al. 2004). Furthermore, mutagenesis would be quite helpful to study reverse genetics and functional genomic studies. The recent molecular and omic techniques could explore at large the induced mutations to study the gene functions & expression and genome by developing suitable molecular markers and genetic maps for precision selection. Sustainable production of alliums focusing only on onion and garlic under changing climatic scenario and to cater burgeoning population, induced mutagenesis along with conventional & modern breeding approaches and other allied sciences like biotechnological tools, physiology and plant protection could be the best and effective option. Owing to low cost and simple procedure, mutagenesis induction in alliums remains a wonderful approach for developing widely adapted cultivars having biotic and abiotic resistance for the sustainable productivity under rapidly changing climate scenario. Thus, it can be said that mutation breeding combined with modern biotechnological tools is very important and relevant for genetic improvement of allium species.

Declarations

Conflict of interest The authors declare that there is no conflict of interest.

References

Agrawal MK, Fageria MS, Dhaka RS (2003) Garlic breeding: a review. Agric Rev 24:70–74

Ahirwar R (2015) Gamma radiation induced chromosomal aberrations at mitosis in *Allium cepa* L. Int J Sci Res 4(4):855–858

Ahirwar R, Verma RC (2016) Ethyl methane sulphonate (EMS) induced translocation and inversion heterozygote in *Allium cepa* L. Cytologia 81(2):149–153

Ahloowalia BS (1998) *In vitro* techniques and mutagenesis for the improvement of vegetatively propagated plants. In: Jain SM, Brar DS, Ahloowalia BS (eds) Somaclonal variation and induced mutations in crop improvement. Springer, Dordrecht, pp 293–309

Ahloowalia BS, Maluszynski M (2001) Induced mutations: a new paradigm in plant breeding. Euphytica 118:167–173

Ahloowalia BS, Maluszynski M, Nichterlein K (2004) Global impact of mutation derived varieties. Euphytica 135:187–204

Ahmad S, Qureshi S (1992) Comparative study of two cultivars of *Zea mays* after seed irradiation. II. Effect of radiation on yield. Sarhad J Agr 8:655–658

Ali BT, Amin BT, Behzad S (2012) Ethyl methane sulphonate (EMS) induced mutagenesis in Malaysian rice (cv. MR219) for lethal dose. Am J Plant Sci 3:1661–1665

Al-Oudat M (1990) Effect of low dose gamma irradiation on onion yield. Ann Biol 6:61–67

Al-Safadi B, Mir AN, Arabi MIE (2000) Improvement of garlic (*Allium sativum* L.) resistant to white rot and storability using γ-irradiation induced mutations. J Genet Breed 54(3):175–181

Al-Safadi B, Sharabi NE, Nabulsi I (2002) Evaluation of tissue culture and growth assays to identify irradiated from non-irradiated vegetables. J Veg Crop Prod 8:109–121

Amjad M, Anjum MA (2002) Effect of gamma radiation on onion seed viability, germination potential seedling growth and morphology. Pak J Agri Sci 39:202–206

Amjad M, Anjum MA (2007) Effect of post-irradiation ageing on onion seeds. Acta Physiol Plant 29(1):63–69

Bado S, Forster BP, Nielen S, Ali AM, Lagoda PJ, Till BJ, Laimer M (2015) Plant mutation breeding: current progress and future assessment. Plant Breed Rev 39:23–88

Banerjee M, Sharma AK (1971) Effects of certain physical and chemical agents on the chromosomes of different varieties of *Allium cepa* L. Ind Agric 15:199–206

Banjare C, Shukla N, Sharma PK, Shrivastava R, Chandravanshi D (2017) Effect of Ethyl Methane Sulphonate (EMS) on sprouting and survival characteristics of garlic (*Allium sativum* L.). Agric Update 12:1350–1356

Bhamburkar S, Bhalla JK (1980) Different mutagenic sensitivity of three variety of *Allium cepa* L. J Cytol Genet 15:178–185

Bhat TA, Khan AH, Parveen S (2005) Comparative analysis of meiotic abnormalities induced by gamma rays, EMS and MMS in *Vicia faba* L. J Indian Botan Soc 84:45–58

Campos FF, Generoso WM, Caprinpin JM (1960) Effect of X-rays on the bulbs and seeds of onion (*Allium cepa* L.). Philipp Agric 44:125–133

Choudhary AD, Dnyanansagar VR (1982) Studies on effect of physical and chemical mutagens in garlic (*Allium sativum* L.). J Indian Bot Soc 61:85–90

Choudhary AD, Dnyansagar VR (1980a) Induced chromosomal aberrations in garlic. J Cytol Genet 15:58–60

Choudhary AD, Dnyansagar VR (1980b) Effect of physical and chemical mutagens on morphological parameters in garlic. J Indian Bot Soc 59(3):202–206

Datta SK (1984) *Radiosensitivity*. Everyman's. Science 19:269–273

Datta SK (2002) Parameters for detecting effects of ionizing radiations on plants. In: Tripathi RD, Kulshreshtha K, Agarwal M, Ahmad KJ, Varshney CK, Krupa S, Pushpangadan P (eds) Plant responses to environmental stress. International Book Distributing Co, Lucknow, pp 257–265

Datta BK, Bhattacharya GN, Sudhendu M (1992) Differential susceptibility of two varieties of *Vigna radiata* in response to ethyl methane sulphonate. In: Proceedings of the environmental pollution: impact of technology on quality of life, Febuary 21–23 Santiniketan, India, pp 25–30

Datta SK, Chakrabarty D, Verma AK, Banerji BK (2011) Gamma ray induced chromosomal aberrations and enzyme related defense mechanism in *Allium cepa* L. Caryologia 64(4):388–397

Devi SJ (2020) Morphological studies of gamma irradiated *Allium tuberosum* Rottler ex Sprengel local cv. Ningtham sidabi. Stud Indian Place Names 40(50):684–692

Dhanayanth KPM, Reddy V (2000) Cytogenetic effects of gamma rays and ethyle methane sulphonate in chilli piper (*Capsicum annuum*). Cytology 65:129–133

Duangjit J, Bohanec B, Chan AP, Town CD, Havey MJ (2013) Transcriptome sequencing to produce SNP-based genetic maps of onion. Theor Appl Genet 126:2093–2101

Eady CC (1995) Towards the transformation of onion (*Allium cepa* L). N Z J Crop Hort Sci 23:239–250

El-Fiki A, Adly M (2020) Morphological, molecular, and organosulphur compounds characterization in irradiated garlic (*Allium sativum*) by GC–MS and SCoT markers. J Radiat Res Appl Sci 13(1):61–70. https://doi.org/10.1080/16878507.2019.1697079

Etoh T, Simon PW (2002) Diversity, fertility and seed production of garlic. In: Rabinowitch HD, Currah L (eds) Allium Crop Science: Recent Advances, CAB Intl. Wallingford, UK, pp 101–117

FAOSTAT (2020) Onion production, area and productivity. http://www.fao.org/faostat/en/#data/QC. Accessed 20 Feb 2021

Fritsch RM, Friesen N (2002) Evolution, domestication and taxonomy. In: Rabinowitch HD, Currah L (eds) Allium Crop Science: Recent Advances. CABI International, UK, pp 5–30

Gohil RN, Koul AK (1984) Investigations on effect of physical mutagen on garlic (*Allium sativum* L.). Allium News 1:70–74

Gore MA, Wright MH, Ersoz ES, Bouffard P, Szekeres ES, Jarvie TP, Hurwitz BL, Narechania A, Harkins TT, Grills GS, Ware DH, Buckler ES (2009) Large-scale discovery of genes enriched SNPs. Plant Genome 2:121–133

Guar BK, Nirale AS (1975) Limited growth stimulation by low dose X-irradiation of onion seedling. Stimul Newsl 8:13–17

Hao WU, Cheng-Zuo LI (2006) Breeding of the new onion variety by laser mutagenesis and culture techniques. Acta Laser Biol Sin 15:651–654

Hassan L, Ahmad SD (2000) Chromosomal aberrations induced by chemical mutagens in allium. Pak J Biol Sci 3(7):1187–1189

Hassan MHA, Wang H, Li X (2014) Performance of garlic callus, regenerated sprouts and formed plantlets under ethyl methane sulphonate affect. J Int Acad Res Multidisc 5(2):446–456

Humera A, Javed I (2012) Genetic analysis of somaclonal variants and induced mutants of potato (*Solanum tuberosum* L.) cv. diamante using RAPD markers. Pak J Bot 44:215–220

IAEA (2020) Mutant varieties database. https://mvd.iaea.org/#!Search?page=1&size=25&sortby=Name&sort=ASC&Criteria[0][field]=FreeText&Criteria[0][val]=allium. Accessed 20 Feb 2021

Ipek ME, Ipek AH, Almquist SG, Simon PW (2005) Demonstration of linkage and development of the first low-density genetic map of garlic, based on AFLP markers. Theor Appl Genet 110:228–236

Ipek M, Sahin N, Ipek A, Cansev A, Simon PW (2015) Development and validation of new SSR markers from expressed regions in the garlic genome. Sci Agric 72:41–46

Jain MS, Suprasanna P (2011) Induced mutations for enhancing nutrition and food production. Gene Conserve 10(41):201–2015

Jankowicz-Cieslak J, Huynh OA, Dussorth B, Saraye B, Till BJ (2012) Low-cost mutation discovery methods suitable for developing countries. Sci Med 3:245–249

Joshi N, Ravindran A, Mahajan V (2011) Investigations on chemical mutagen sensitivity in onion (*Allium cepa* L.). Int J Bot 7(3):243–248. https://doi.org/10.3923/ijb.2011.243.248

Joung JK, Sander JD (2013) TALENs: a widely applicable technology for targeted genome editing. Nat Rev Mol Cell Biol 14:49–55. https://doi.org/10.1038/nrm3486

Kamenetsky R (2007) Garlic: Botany and Horticulture. Hortic Rev 33:123–171. https://doi.org/10.1002/9780470168011.ch2

Kataria AS, Singh N (1989) Mutation studies in onion (*Allium cepa* L.) II. Mutagenic effectiveness and efficiency of gamma rays, EMS, NMU and EL. Ann Agric Res 10:131–135

Kataria AS, Singh N (1989) Mutation studies in onion (*Allium cepa* L.) III. Types and frequency of macromutations. Ind J Hort 46(3):395–400

Kataria AS, Singh N (1989) Mutation studies in onion (*Allium cepa* L.) I. Mutagen sensitivity and mutability. Ind J Hort 46(2):199–203

Kataria AS, Singh N (1990) Mutation studies in onion (*Allium cepa* L.) IV. Effect of mutagens on total soluble solids contents. Ind J Hort 47(2):216–219

Kato M, Masamura N, Shono J, Okamoto D, Abe T, Imai S (2016) Production and characterization of tearless and non-pungent onion. Sci Rep 6:23779. https://doi.org/10.1038/srep23779

Kaul A (1985) Induction of somatic mutations in *Allium sativum* and *Allium cepa* var. *viviparum*. Dissertation, University of Kashmir, India

Khan S, Al-Qurainy F (2009) Mutagenic effect of sodium azide on seed germination of *Eruca sativa* (L.). Aust J Basic Appl Sci 3:3081–3087

Khan S, Goyal S (2009) Improvement of mungbean variety through induced mutations. Afri J Plant Sci 3:174–180

Khar A, Hirata S, Abdelrahman M, Shigyo M, Singh H (2020) Breeding and genomic approaches for climate-resilient garlic. In: Kole C (ed) Genomic designing of climate-smart vegetable crops. Springer, Cham. https://doi.org/10.1007/978-3-319-97415-6_8

Kharkwal MC (1998) Induced mutations in chickpea (*Cicer arietinum* L.) I. Comparative mutagenic effectiveness and efficiency of physical and chemical mutagens. Indian J Genet 58(2):159–167

Kharkwal MC (2012) A brief history of plant mutagenesis. In: Shu QY, Forster BP, Nakagawa H (eds) Plant mutation breeding and biotechnology. CABI, Wallingford, pp 21–30

Kharkwal MC, Shu QY (2010) The role of induced mutations in world food security. In: Shu QY (ed) Induced plant mutations in the genomics era. Food and Agriculture Organization of the United Nations, Rome, pp 33–38

Khassanov FO (2018) Taxonomical and Ethnobotanical Aspects of *Allium* Species from Middle Asia with Particular Reference to Subgenus *Allium*. In: Shigyo M, Khar A, Abdelrahman M (eds) The Allium Genomes. Compendium of Plant Genomes. Springer, Switzerland, pp 11–22. https://doi.org/10.1007/978-3-319-95825-5_2

Kim DW, Jung TS, Nam SH, Kwon HR, Kim A, Chae SH, Choi SH, Kim DW, Kim RN, Park HS (2009) GarlicESTdb: an online database and mining tool for garlic EST sequences. BMC Plant Biol 9(1):61. https://doi.org/10.1186/1471-2229-9-61

Kirtane SA (2014) Studies on induced mutations in onion: effect of combination treatments of gamma radiation and sodium azide on biological and biochemical parameters of onion (*Allium cepa* L). AARJMD 1(25):119–129

Kirtane SA (2014) Studies on Induced mutations in onion: frequency and spectrum of chlorophyll mutations. Biol Forum 6(2):141–144

Kirtane SA (2018) Comparative mutagenic effectiveness and efficiency of sodium azide and gamma radiation in onion (*Allium cepa* L.). Int J Theor Appl Sci 10(1):169–173

Kirtane S, Dhumal KN (2004) Studies on induced mutations in onion: biological and cytological effects of mutagen in M_1 generation. Int J Mendel 21:11–13

Kirtane S, Laware SL, Khar A, Lawande KE, Dhumal KN (2000) Induced macromolecular variability in onion (*Allium cepa* L). In: National symposium on Onion and Garlic, production and post-harvest management, challenges and strategies. November 19–21. Krishi Vigyan Kendra, YCMOU, Nashik (MS), India, p 186

Kirtane S, Lawande KE, Trivedi AP, Dhumal KN (2001) Pollen sterility in mutagens treated onion (*Allium cepa* L). Allium Improv Newsl 11:4–6

Koul AK, Gohil RN (1971) Further studies on natural triploidy in viviparous onion. Cytologia 36:253–261

Kostov K, Batchvarova R, Slavov S (2007) Application of chemical mutagenesis to increase the resistance of tomato to *Orobanche ramosa* L. Bulg J Agric Sci 13:505–513

Kulkarni GB (2011) Effect of mutagen on pollen-fertitity and other parameter in horse gram *Imacrotyloma untflorum* (lam). Biosci Discov 2:146–150

Kumar S, Chaudhary BD (1996) Effect of doses of physical and chemical mutagens on the germination and mortality/survivability of induced mutations in Indian mustard (*Brassica juncea* (L.) Czern and Coss). Agr Sci Digest 16:17–20

Kumar DS, Chakrabarty D, Verma AK, Banerji BK (2011) Gamma ray induced chromosomal aberrations and enzyme related defense mechanism in *Allium cepa* L. Caryologia 64(4):388–397

Kuhl JC, Cheung F, Yuan Q, Martin W, Zewdie Y, McCallum J, Catanach A, Rutherford P, Sink KC, Jenderek M, Prince JP (2004) A unique set of 11,008 onion expressed sequence tags reveals expressed sequence and genomic differences between the monocot orders Asparagales and Poales. Plant Cell 16:114–125

Lawande KE, Khar A, Mahajan V, Srinivas PS, Sankar V, Singh RP (2009) Onion and garlic research in India. J Hort Sci 4(2):91–119

Lee IS, Kim DS, Hyun DY, Lee SJ, Song HS, Lim YP, Lee YI (2003) Isolation of gamma-induced rice mutants with

increased tolerance to salt by anther culture. J Plant Biotechnol 5:51–57
Ma K, Kwag J, Zhao W, Dixit A, Lee G, Kim H, Chung M et al (2009) Isolation and characteristics of eight novel polymorphic microsatellite loci from the genome of garlic (*Allium sativum* L.). Sci Hortic 122:355–361
Mahajan V, Devi A, Khar A, Lawande KE (2015) Studies on mutagenesis in garlic using chemical mutagens to determine lethal dose (LD50) and create variability. Ind J Hortic 72(2):289–292
Mahandjiev A, Kosturkova G, Mihov M (2001) Enrichment of *Pisum sativum* gene resources through combined use of physical and chemical mutagens. Israel J Plant Sci 49:279–284
Martin W, McCallum J, Shigyo M, Jakŝe J, Kuhl JC, Yamane N, Sink KC, Town CD, Havey MJ (2005) Genetic mapping of expressed sequences in onion and in silico comparisons show scant collinearity with rice. Mol Genet Genom 274:197–204
McCallum CM, Comai L, Greene EA, Henikoff S (2000) Targeted screening for induced mutations. Nat Biotechnol 18:455–457
McCallum CM, Comai L, Greene EA, Henikoff S (2000) Targeting induced local lesions in genomes (TILLING) for plant functional genomics. Plant Physiol 123:439–442
Mlcochova L, Chloupek O, Uptmoor R, Ordon F, Friedt W (2004) Molecular analysis of the barley cv. 'Valticky' and its X-ray-derived semi dwarf-mutant 'Diamant.' Plant Breed 123:421–427. https://doi.org/10.1111/j.1439-0523.2004.01023.x
Mostafa HH, Wang H, Shen D, Qiu Y, Li X (2015) Sprout differentiation and mutation induction of garlic (*Allium sativum* L.) callus exposed to gamma radiation. Plant Growth Regul 75(2):465–471. https://doi.org/10.1007/s10725-015-0060-z
Nabulsi I, Al-Safadi B, Ali NM, Arabi MIE (2001) Evaluation of some garlic (*Allium sativum* L.) mutants resistant to white rot disease by RAPD analysis. Ann Appl Biol 138(2):197–202. https://doi.org/10.1111/j.1744-7348.2001.tb00102.x
Oladosu Y, Mohd YR, Norhani A, Ghazali H, Asfaliza R, Harun AR, Gous M, Magaji U (2016) Principle and application of plant mutagenesis in crop improvement: a review. Biotechnol Biotechnol Equip 30(1):1–16. https://doi.org/10.1080/13102818.2015.1087333
Park SJ, Sook K, JaeSung K, JaeMoon H, SoonTae K (2004) Effect of γ-radiation treatment on growth and antioxidative capacity in garlic (*Allium sativum* L.). Korean J Horti Sci Tech 22(4):407–410
Peiwen X, Sun H, Yang Y, Liu H, Sun S (1999) A primary study on mutation induction of *in vitro* cultured garlic shoots by 60Co γ-ray irradiation. Acta Agri Nucleatae Sin 13:142–146
Perez-Moreno L, Lopez-Munoz J, Pureco A, Hinojosa JC (1991) Production of radiation induced mutants of garlic (*Allium sativum* L.) resistant to white rot caused by the fungus *Sclerotium cepivorum* Berk. Plant Mutat Crop Improv IAEA 498:211–219
Predieri S, Divrgilio N (2007) *In-vitro* mutagenesis and mutant multiplication, Chapter 30. In: Jain SM, Haggman H (eds) Protocols for micropropagation of woody trees and fruits. Springer, Berlin, pp 323–333
Ramakrishnan U (2002) Prevalence of micronutrient malnutrition worldwide. Nutr Rev 60:S46-52
Robledo-Paz A, Tovar-Soto HM (2012) Biotechnological tools for garlic propagation and improvement. Innov Biotechnol 10:30636. https://doi.org/10.5772/30636
Rosario TL, Miranda MB (1991) Induced mutation in garlic (*Allium sativum*). In: Plant mutation breeding for crop improvement, vol 1. IAEA, Vienna, pp 485–489
Schunk CR, Eberius M (2012) Phenomics in plant biological research and mutation breeding. In: Shu QY, Forster BF, Nakagawa H (eds) Plant mutation breeding and biotechnology. CABI, UK, pp 535–559
Schwartz HF, Mohan SK (1995) Compendium of onion and garlic diseases. Disease compendium series of the American Phytopathological Society
Selvaraj N, Natarajan S, Ramaraj B (2011) Studies on induced mutations in garlic. Newsletter 45:40–41
Shashidhar TR, Dharmatti PR, Nagaraja TE (2005) Determination of LD50 for physical mutagen in garlic cv. Vannur Local Karnataka J Hort 1(2):110–111
Sigurbjörnsson B, Micke A (1974) Philosophy and accomplishments of mutation breeding. Polyploidy and induced mutations in plant breeding. IAEA, Vienna, pp 303–343
Sikora P, Chawade A, Larsson M, Olsson J, Olsson O (2011) Mutagenesis as a tool in plant genetics, functional genomics, and breeding. Int J Plant Genom. https://doi.org/10.1155/2011/314829
Singh BB (1974) Radiation induced changes in catalase, lipase and ascorbic acid of safflower seeds during germination. Radiat Bot 14:195–199
Singh F, Khoshoo TN, Ved BS (1967) Natural triploidy in viviparous onions. Cytologia 32:403–407
Singh H, Verma P, Lal SK, Khar A (2021) Optimization of EMS mutagen dose for short day Indian onion (*Allium cepa* L.). Ind J Hort 78(1):35–40
Sinuraya M, Rosmayati R, Hasanuddin H, Hanafiah DS (2015) Radio-sensitivity and the influence of gamma rays irradiation on local samosir shallots. In: Proceedings of the Annual International Conference Syiah Kuala University, vol 5, pp 228–231
Stadler LJ (1928) Mutations in barley induced by X-rays and radium. Science 68:186
Taner Y, Besirli G, Kunter B, Yanmaz R (2004) Determining effective radiation mutagen dose for garlic (*Allium sativum* L). Bahce (yalova) 33(1–2):95–99
Till BJ, Reynolds SH, Weil C et al (2004) Discovery of induced point mutations in maize genes by TILLING. BMC Plant Biol. https://doi.org/10.1186/1471-2229-4-12
Till BJ, Cooper J, Tai TH et al (2007) Discovery of chemically induced mutations in rice by TILLING. BMC Plant Biol 7:19. https://doi.org/10.1186/1471-2229-7-19
Ussuf KK, Nair PM (1974) Effect of gamma irradiation on the indole acetic acid synthesizing system and its significance in sprout inhibition of potatoes. Rad Bot 14:251–256
Van Der Meer QP (1997) Old and new crops within edible Alliums. Acta Hort 433:17–31
Van Harten AM (1998) Mutation breeding. Theory and practical applications. Cambridge University, Cambridge, p 353

Wani MR, Khan S (2006) Estimates of genetic variability in mutated populations and the scope of selection for yield attributes in *Vigna radiata* (L.) Wilczek. Egypt J Biol 8:1–6

Watanabe Y, Yukawa M, Kim HS, Nishimura Y (2000) Radiation effects on growth and seed germination of Arabidopsis. Annual Report 1999–2000. National Institute of Radiological Sciences, Anagawa, Chiba-shi, Japan

Wattoo JI, Aslam K, Shah SM, Shabir G, Sabar M, Naveed SA, Waheed R, Samiullah QH, Muqaddasi AM (2012) Ethyle methane sulphonate (EMS) induced mutagenic attempts to create genetic variability in Basmati rice. J Plant Breed Crop Sci 4(7):101–105. https://doi.org/10.5897/JPBCS11.080

Xixiang L, Hassan MHA, Haiping W (2014) Performance of garlic callus, regenerated sprouts and formed plantlets under ethyl methane sulphonate affect. J Int Acad Res Multidisc 5(2):446–456

Yamaguchi H, Nagatomi S, Morishita T et al (2003) Mutation induced with ion-beam irradiation in rose. Nucl Instr Methods Phys Res 206:561–564. https://doi.org/10.1016/S0168-583X(03)00825-5

Zargar GH, Zeerak NA, Ahanger HU, Wani SA (1994) Mutagenic effect of gamma rays, ethyl methanesulphonate and their combination in top-onion (*Allium cepa* var. viviparum). J Nucl Agric Biol 23(4):249–250

Zewdie Y, Havey MJ, Prince JP, Jenderek MM (2005) The first genetic linkages among expressed regions of the garlic genome. J Am Soc Hortic Sci 130:569–574

Zhao WG, Chung JW, Lee GA, Ma KH, Kim HH, Kim KT, Chung IM, Lee JK, Kim NS, Kim SM, Park YJ (2011) Molecular genetic diversity and population structure of a selected core set in garlic and its relatives using novel SSR markers. Plant Breed 130:46–54

Zheng Z, Wang HB, Chen GD, Yan GJ, Liu CJ (2013) A procedure allowing up to eight generations of wheat and nine generations of barley per annum. Euphytica 191:311–316. https://doi.org/10.1007/s10681-013-0909-z