Review Article

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Mutation breeding in chickpea

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Sanjeev Kumar,[1] Gopal Katna,[2] Neha Sharma[1]
[1]Advanced Centre for Rainfed Agriculture (ACRA) Dhiansar, SK University of Agriculture Sciences & Technology (SKUAST-J), India
[2]Department of Crop Improvement, CSK HPKV, India

Correspondence: Sanjeev Kumar, Advanced Centre for Rainfed Agriculture (ACRA) Dhiansar, SK University of Agriculture Sciences & Technology (SKUAST-J), Jammu-181 133, India, Email ssalgotr@gmail.com

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Introduction

The use of induced mutations has played a key role in the improvement of superior plant varieties. A large number of improved mutant varieties have been released for commercial cultivation in different crop species.[1,2] The history of mutation research dates back to 1900 to 1927 when the concepts of mutation and mutation rates were developed and with the discovery of mutagenic action of X-rays in 1927, an efficient tool for probing mutation in nature was obtained. By the end of Second World War the chemical mutagens came in light but after the discovery of DNA the action of mutagens and mechanism were clearly understood and used for the crop improvement. Bateson[3] put forward the presence absence theory, according to which all mutations are due to losses of normal genes later revived considering the position effect of rearrangements leading to new phenotypes. Muller in 1928 stressed that the gene has one unique property without which evolution could not have taken place and introduced the concept of mutation rate. Treatment with mutagens alters genes or breaks chromosomes. Gene mutations occur naturally as errors in DNA replication. Most of these errors are repaired, but some may pass the next cell division and become established in the offspring as spontaneous mutations. Mutations observed in a particular gene are rare and every plant may carry one or more spontaneous mutations into the next generation. Gene mutations without phenotypic (visible) expressions are usually not recognized. Consequently, genetic variation appears rather limited, and scientists have to resort to mutation induction. In ancient high breed cultivated plants a productive variety with additional promising characteristics can thus be produced without the necessity of back-crossing. In recently cultivated plants new characteristics can be introduced, linkage can be broken and little segments of a chromosome can be transferred to homologous chromosome. Not all the undesirable mutations useless for breeding work. Combined with favourable ideotype they may become variable for breeding purpose and pathological mutants useless for breeding work can be used for theoretical studies of different kind.[4] Mutation breeding is a proven supplement and an effective substitute to conventional breeding where only specific improvement in a variety is required without significantly affecting its acceptable phenotype.

Pulses are second in importance to human and animal diets after cereals contributing significantly to global food and nutritional security. Among the pulses, chickpea (*Cicer arietinum* L.) is one of the most widely grown legume crop ranking second in area and third in production. Being a rich and cheap source of protein, chickpea can help people to improve the nutritional quality of their diet and thus plays a crucial role in food security in developing countries. Currently the productivity of chickpea is very low and has been stagnant in recent years. Despite high morphological variability, genetic variation in chickpea is limited probably due to its monophyletic descendence from *Cicer reticulatum*.[5–7] In the past, the staple cereal crops, especially wheat, rice and maize, have received highest research priorities; consequently, considerable yield improvements were made in these crops. In contrast, legumes are under researched compared to cereals. Chickpea is cultivated mostly in marginal lands under rainfed conditions, with low and unstable productivity. Its production is adversely affected by several biotic and abiotic stresses. Ascochyta blight, botrytis gray mold, fusarium wilt, and dry root rot; pod borer (*Helicoverpa armigera* L.); drought, heat, salinity and water logging are important stresses potentially limiting the productivity of chickpea worldwide. A narrow genetic base, due to the bottlenecks associated with its evolution and domestication, as well as due to the replacement of locally adapted crop landraces by the genetically advanced modern varieties, is the main reason behind the lagoona. Realizing the significance of legumes in improving nutrition and livelihood of poor farmers, there is a need to breed new crop cultivars with a broad genetic base, capable of withstanding frequent climatic fluctuations as well as resistance/tolerance to biotic and abiotic stresses. Exploitation of new and diverse sources of variation is needed for the genetic enhancement of legumes. The more important factors in producing variability in plants are hybridization, recombination and mutation (spontaneous and induced). Seed setting in chickpea through hybridization during harsh environments in its growing areas is difficult and even some times impossible. Thus, creation of genetic variability through induced mutation is a suitable procedure to evolve better cultivars with improved agronomic traits like seed size and seed yield.[8–11] Wild relatives with enhanced levels of resistance/tolerance to multiple stresses provide important sources of genetic diversity for crop improvement. However, their exploitation for cultivar improvement is limited by cross-incompatibility barriers and linkage drags. The naturally occurring mutation rate is too low for practical application. Therefore, physical and chemical mutagens are useful for increasing the mutations rate.

A mutant variety is a new plant variety that is bred through either:

a. Direct use, involving use of mutant line itself as a variety
b. Indirect use, which involves using mutant line as a parental variety in crossbreeding (cross between mutant lines or with a commercial variety)
c. Using mutant gene allele (trait)
d. Using wild species genes translocated into plant genomes through irradiation/mutagen- derived translocations
e. Mutations are classified into two groups depending upon the magnitude of phenotypic effect produced by them Gaul[12]
f. Macro mutations: These produce a large recognizable phenotype effect on individual plants. These are oligogenic in nature and can be easily selected in the M_2 generation.
g. Micro mutations: These produce a small phenotypic effect that can be identified only on the basis of a population. These are

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polygenic in nature and selection for such mutations can be delayed till M_3 or later generations.

Mutagens

Several types of mutagenic agents are used extensively to create genetic variation for use in genetics and/or crop improvement according to the response expected. Physical and chemical mutagens cause DNA damage, initiate different mechanisms either by killing the damaged cell or by repairing DNA lesions. The consequences of these processes are directly linked to mutation breeding. The most commonly used mutagens are

a. Physical: X-rays, Gamma rays, UV radiations, β-particles, Neutrons and Particles from accelerations

b. Chemical: Base analogues, Antibiotics, Alkylating agents, Acridines, Azides, Hydroxylamine and Nitrous acid.

Despite the availability of a range of mutagenic agents, it is still difficult to direct the induction of mutation with desired expression of the character. Britt[13] provided an overview on changes that can occur at gene, chromosome, and genome levels, including chromosomal break down, inversion, duplication, translocation, and point mutation.

Physical mutagen

Ionising radiations including X-ray, gamma (γ) rays, neutrons and high-energy ion beams can cause double-strand DNA breaks. The γ-ray (obtained generally from radioactive isotope of cobalt 60) cause point mutations and small deletions. Among the physical mutagens, gamma rays are the most popular among mutation breeders because of the convenience of use and their ability to penetrate deep into a biological matter. The UV radiations possess limited tissue penetrating ability as a result of which their use is restricted to pollen grains and plant cells/protoplasts. The radiation dose is determined by the intensity of radiations and length of explosive. It is expressed in Roentgen (R) units, which are a measure of the number of ionizations that occur. In the mutation breeding experiments, irradiation dose is generally expressed as kR or Gray (Gy) where 1 Gy=100 rad and 1 kR=10 Gy. The unit of absorbed dose is rad (radiation absorbed dose) where 1 rad=100 erg/g=10-2 joule/kg and it is expressed as rad per second or per minute or per hour. Gamma-rays induce nucleotide substitutions and small deletions of 2–16 bp with the mutation frequency of about one mutation/6.2 Mb.[14] Fast neutron bombardment causes translocations, chromosome losses and large deletions and is believed to result in kilobase-scale deletions. Ion beams are usually generated by particle accelerators, e.g. cyclotrons, using ^{20}Ne, ^{14}N, ^{12}C, ^{7}Li, ^{40}Ar or ^{56}Fe as radioisotope sources.[15] They are responsible for linear energy transfer (LET), and as LET increases, higher biological effects such as lethality, chromosomal aberration, etc., are induced as compared to most commonly used physical mutagens.[16] The LET for gamma rays and X-rays accounts in the range of 0.2–2 keV/µm and hence is called low-LET radiations. In contrast the high-LET radiations from carbon (23 keV/µm) and iron (640 keV/µm) ion beams provide much larger and wider ionisation energy. High-LET ion beam radiations cause more localised, dense ionisation within cells than those of low-LET radiations (Abe et al. 2012). An ideal irradiation dose is a dose at which ion beams show the highest mutation rate at any locus of interest.[15] Hence, irradiation doses should be chosen by testing different doses at a time and screening the irradiated population for desired mutants for which traits such as survival rate, growth rate, chlorophyll mutation, and so on, which are early indicators for occurrence of mutation are used by the researchers.[15] Energetic heavy-ion beams are used for generating mutants in higher plants because these induce mutations with high frequency at a relatively low dose (i.e. at which virtually all plants survive) and thereby induce a broad spectrum of phenotypes without affecting other plant characteristics.[15] Advantages of ion beam mutagenesis include low dose with high survival rates, induction of high mutation rates and wide range of variation. Gamma rays and fast neutrons have been effectively used in obtaining chickpea mutants[17] in Kabuli and Desi cultivars of chickpea. Ambarkar et al.[18] studied the visible mutations in the gamma irradiated chickpea cultivar and found 20 kR most effective. Bhardwaj & Sood[19] observed that the effect of irradiation on the segregating generation is producing heritable variations but appears to be balanced out with no net gain or loss in the total phenotypic variability.

Chemical mutagens

Chemical mutagens include base analogues, acridine dyes, nitrous acid, hydroxylamine, etc. which has high mutation rates and induce mostly point mutations. The most commonly used include, ethyl methanesulphonate (EMS), diethyl sulphate (DES), methyl nitroso urea (MNH), ethyl nitroo urea (ENH), ethyleneimine (EI) all of which belong to a special class of alkylating agents. All these chemicals react with DNA by alkylating the phosphate groups and also the purine and pyrimidine bases or reacts with guanine or thymine by adding an ethyl group which causes the DNA replication machinery to recognise the modified base as an adenine or cytosine, respectively. Chemical mutagenesis induces a high frequency of nucleotide substitutions, and a majority of the changes (70–99 %) in EMS-mutated populations are GC to AT base pair transitions.[20,21] Sodium azide (Az) and methylnitrosourea (MNU) are also used in combination. Az-MNU mutagenesis can induce a shift in either direction of GC to AT shifts or AT to GC shifts.[21] The dose of a chemical mutagen mainly depends upon concentration, duration of treatment and temperature during treatment. Modifying factors are pre-soaking, pH of solution, metallic ions, carrier agents, post washing, post drying and storage of seeds. All these chemical mutagens are strongly carcinogenic and extreme care should be taken while handling and disposal. EMS is an IARC group 2B carcinogen. Working with MNU can be sometimes difficult as it is unstable above 20 °C. There are three factors important to the success of mutation breeding[22]

a. The efficiency of mutagenesis;

b. The starting plant material;

c. The mutant screening.

Thus, a new crop variety can be obtained by selecting a mutant with modification to a target trait while retaining the existing valuable ones. However, stepwise trait improvement is expected to be especially effective for plants, those which cannot be crossbred and/or those which have highly duplicated genomes. The most commonly used chemical mutagens in chickpea breeding are EMS, SA and HZ. Chemical mutagens have been found to be more effective in causing mutations than the physical mutagens as reported by Kharkwal[23,24] and Shah et al.[25] in chickpea. Bhatt et al.[26] studies the effect of EMS, SA and HZ on two varieties of chickpea viz., Avrodhi and BG-256 and obtained a linear and dose dependent decrease in germination, pollen fertility and survival of all the mutagenic treatments. Khan et al.[27] found that the frequency and spectrum of morphological mutants was relatively wide with EMS treatments followed by HZ and SA. The mutant types included dwarf, compact, prostrate, gigas, white flower, non-flowering and sterile.

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Choice of plant material for mutagenic treatment

Mutation breeding programmes should be clearly planned and well defined and large enough to select desirable mutations as the low frequencies likely to be encountered. The plant materials such as seeds in the case of seed-propagated crops are exposed to mutagen(s). Purity of the parental material used for mutagenesis is extremely important. Tissues that are metabolically active or have high water content are more sensitive to radiation damage.[28] The dose rate or intensity, type of mutagen and concentration of mutagen to be used may vary depending upon the type of material chosen. Although mutations can be induced in all types of plants, to obtain desired mutant in prescribed time, effort and facilities, it is important to consider suitability of the plant species for mutation breeding. Self-pollinating, seasonal plants are more suitable as they can be grown in large numbers in smaller field area and generation time is shorter making chickpea a better candidate for mutation breeding. Thus, mutants can be identified and confirmed in shorter time periods. The genotypes give differential response to mutagens; hence two or more varieties must be taken for mutagenesis. It is always beneficial to select a well-adapted high yielding variety for improving one or two specific traits. Plants with higher levels of ploidy may continue to segregate before the mutant phenotype is identified and stabilised. One of the bottlenecks of plant mutation breeding is the occurrence of chimeras following the mutagenic treatment of multicellular tissues.

Population size for mutagenesis

Mutation is a chance event thus larger experimental population is recommended in early generations. Monogenic trait in a diploid species is easy to be identified. However, in case of quantitative (polygenic) traits and in plants with higher ploidy, observing a mutant is possible if the mutagenised population is sufficiently large. As mutation occurs at a frequency of one in thousand plants thus at least 1,000 M_2 generation plants will have to be screened to attain the statistical probability which can be obtained from atleast 20 M_1. To ensure that the mutant occurs, it is recommended that a higher number of seeds are irradiated. Also, if several mutants of a particular phenotype are identified, it presents an opportunity to select and carry forward the one with the potential for the best economic returns.

Dose of mutagen

The dose of the mutagen should be high enough to increase the probability of inducing a mutation but below the level of causing damage to the cells/tissues resulting to lethality. In case of seed treatment, the dose that is sufficient to inhibit about 50% germination, i.e. LD 50, is generally used to get good results. Generally, irradiated populations are generated by using an LD 50 dose treatment and with a dose lower than LD 50. Since induction of mutation is a chance event, and recovery of a mutation is dependent upon chance of the survival of that individual plant, this strategy improves the probability of obtaining a desirable mutant. The dose and the rate, i.e. duration of application of a mutagen vary with plant species and should be determined through experimentation. In a case where LD 50 dose is already reported, it can be used as a guideline; otherwise, it can be determined by exposing different subsamples of the target plant material (seeds) to a range of doses (low to high) and monitoring survival of the plants in fi eld (up to flowering or maturity). In plants which are sensitive to radiation, doses lower than LD 50 are also used to reduce the mutation load.[29] One of the first steps in mutagenic treatment is the estimation of the most appropriate dose to apply. The common procedure in assessing the most appropriate dosage is based on radio sensitivity, which is estimated through the response of the irradiated material. Radio sensitivity varies with the species and the cultivar, with the physiological condition of plant and organs, and with the manipulation of the irradiated material before and after mutagenic treatment. Correlations between the physiological status of plants and their radio sensitivity are often correlated to water content of the tissue, since the most frequent primary target of ionizing radiation is the water molecule.[30] Chemical mutagen dose is determined in account of the properties of the mutagen (half-life, penetrability, solubility, toxicity or reactivity); type and condition of the treated material before, during and after treatment; interaction with target tissue and culture medium; pH of the medium; and post treatment handling of the material.[31] Chickpea where is seed coat is hard and obstructs tissue penetrability of the mutagen, pre-soaking and prolonged treatment at lower concentrations in combination with the right temperature are practised. The dose may also depend on the genome size of the plant species under study and often negatively reciprocate to the genome size. The combined treatment of physical and chemical mutagens is of apparent interest to a mutation breeder with an intention of enhancing mutation spectrum and frequency, thereby maximising efforts to obtain positive results. In practice, seeds are exposed to physical mutagen first, followed by a treatment of chemical mutagenen in solution.[31] Wani[32] studied the effect of combined treatment of gamma rays and EMS on chickpea cultivar and found that combined treatments were more effective and the effectiveness was high under the intermediate doses. Kamble et al.[33] also found the combination treatment of gamma-rays and EMS more effective in chickpea.

Mutagen-treated seeds are sown in field to obtain their first generation (M_1). Although a mutation event takes place in a cell in the germ part of the seed, it is in a heterozygous condition. Most mutations are recessive in nature and are not expressed in the first generation. M_1 generation generally lethality at various stages of growth and development and its significance is as a source for M_2 generation and ensuring maximum survival of M_1 generation plants is beneficial. Therefore, M_1 plants are numbered and harvested individually. The M_2 plants are often space planted to allow full expression of each individual plant. After meiosis in the M_1 plant, some seeds are formed by fusion of male and female gametes which are carriers of the same mutation and thus are homozygous for the mutation. These seeds give rise to an individual in which the mutant phenotype can be observed. The M_2 generation contains different kinds of visually detectable mutants, however many types of mutations which may be in homozygous condition but cannot be observed by unaided eye.[34] Omar & singh[35] exposed two ascochyta resistant lines to 40 kR, 50 kR and 60 kR and observed that the survival reduced with the increasing dose in chickpea cultivar. Calicius[36] found that doses between 0 Gy and 50 Gy stimulated most of the growth and development process in the seeds whereas doses between 275 Gy and 325 Gy were half lethal on chickpea seeds.

Screening mutants

Since a large number of individual plants have to be screened in the M_2 generation, a rapid and economical method is necessary. The morphological mutants are easy to screen but sufficiently large population is required. The parent variety rows may be introduced

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after certain number of rows of the M_2 population. In case of selection of a disease-resistant mutant, it is necessary that the M_2 population is grown at a hot spot location where the disease is present all the time, otherwise spray of inoculum may be carried out. Rows of susceptible parent variety are must for proper comparison and spreading of the disease. Depending upon the nature of the trait of interest, screening methodology can be employed. All plants showing absence of disease or resistance reaction should be tagged. The M_1 plant corresponding to the M_2 row is marked, and the remaining seeds of the specific M_1 plant may be sown to recover more mutants. All plants with resistant reaction are putative mutants. The plants are allowed to self-pollinate. Seeds from individual plants are collected and each individual plant harvest is given an identification number. In the subsequent season, these have to be sown as plant-to-row progeny (M_3 generation. Often, mutation events are complex, and segregation may not show any mutations in the M_2 generation but in subsequent generations, such as M_3 or M_4, the mutation may appear. The mutant with resistance is grown for few generations in the location where disease occurs while practicing single-plant selection till a completely homozygous progeny is obtained. The resistant mutant may or may not have the ability to out yield the parent variety in the absence of the disease; however, it must show significant yield advantage in the presence of the disease. Agronomic performance of the mutant can be improved by using the mutant in a back-crossing or crossbreeding programme. Kharkwal[37] Rheenen et al.[38] Shah et al.[39] Bhat et al.[26] and many others used chlorophyll mutants as the visible depiction of the induced mutagenesis in the chickpea observing albino, xantha, viridis and chlorine in M_2 and M_3 generations. Screening of mutants of chickpea derived by treatment with EMS was done by at M_4 for nitrogenase activity in the field at three stages *viz.,* vegetative, flowering and pod development depicted large variation in nitrogenase activity in all three stages and also differed significantly in total dry matter, grain yield and nitrogen content of the seeds.[40]

Rheenen et al.[41] treated the desi chickpea cv. Chafa with different doses of EMS for varying duration and observed that 0.10% and 4 hour treatment gave the highest number of mutations which included pale-green foliage, prostrate habit, entire leaflet margin, acuminate leaflet shape, narrow leaflets, brachytic leaves, large leaves, fewer leaflets, rectangular vexillum, open flowers, dwarfing, thick stems, upright canopy, flattened pods, twin pods, large pods, glabrous stems and increased seed yield. Rheenen et al.[42] exposed chickpea seeds ICCV6 cultivar to various doses of gamma radiation and obtained a single plant in M_2 with determinate growth and female sterility. Fasciated mutant characterized by broadened and flattened stem, irregular leaf arrangement and clustering of pods at the stem tip (designated JGM-2) was introduced in chickpea by Gaur and Gaur[43] from M_2 derived 0.40% EMS treated seeds obtained through screening of seeds treated with different doses of EMS. This mutant was observed to have delayed maturity, large seed size and less yielding as compared to its parental cultivar (JG-315). Atta et al.[44] studied the morphological mutants of EMS and gamma-rays induced chickpea mutants and identified mutants in flower colour. Gaur and Gaur[43] identified the broad-few-leaflet mutant which showed a cluster of three to five overlapping leaflets at the terminal end and outwardly curved wing mutants in the EMS treated chickpea mutants. The gene for broad-few-leaflets was designated 'bfl' and the gene for outwardly curved wings was designated 'ocw'. Barshile et al.[11] while studying the effect of different doses of EMS, gamma rays and SA on two chickpea cultivars Vijaya and Vishvas suggested decrease in germination and survival at maturity while seedling injury and pollen sterility increased with increase in concentration in Vijaya and increased frequencies of chlorophyll mutations in Vishvas. Barshile et al.[11] on inducing variation in chickpea through SA, EMS and gamma radiations obtained different type of chlorophyll mutations (chlorina and xantha), leaf mutations (round, curly, gigas, compact and narrow), pod mutations (large, long, small roundish, narrow elongated and small), seed mutations (wrinkled, bold, dark brown and bold dark brown), flower mutations (2 tier and open) and morphological mutations (bold seeded, small pod, early and tall) in the M_2 generation. Shah and Atta[44] observed significant increase in the oil content of pink stem and large leaf mutants of C44 and Pb-1, respectively, while small leaf mutants of desi X Kabuli introgression genotype CH40/91 was significantly high. They also observed that early type mutants in Pb2000 and desi X Kabuli introgression genotype had high palmitic acid, stearic acid, oleic acid and linoleic acid, which supports their role of tolerance against frost. Tolga et al.[45] obtained two induced mutations conferring open flower and determinate growth habit with small leaf characteristics and its progenitor were discovered in M_2 generation on irradiating cultivated chickpea with gamma-rays. These mutant chickpeas were found to be female sterile. Inheritance study showed that the female sterility in the induced mutants was controlled by a single recessive gene (fs).

High-throughput mutation detection and screening techniques

DNA molecular markers

DNA marker techniques can also be used widely in research on plant mutation breeding and genetics for increasing both efficiency and efficacy of the mutation techniques they can be used for tracing the pedigree of induced mutants and tagging important mutations. Consequently, closely linked markers of mutant traits can be used for marker assisted selection (MAS), pyramiding and cloning of mutant genes. At ICRISAT, Buhariwala et al.[46] have developed an EST library from two very closely related chickpea genotypes (Cicer arietinum). A total of 106 EST-based markers were designed from 477 sequences with functional annotations and tested on C. arietinum. TILLING (Targeting Induced Local Lesion In Genomes), a novel, reverse genetics approach that combines advantages of point mutations provided by chemical mutagenesis, with advantages of PCR-based mutant screening, has been introduced.[47] EMS is considered useful as high frequency of single-nucleotide mutations is induced and distributed randomly throughout the genome.[48] TILLING allows EMS-induced G:C to A:T transition point mutation detection and enables recovery of a range of alleles including knockouts and missense changes. TILLING protocol includes PCR amplification of a target DNA fragment of interest from pooled DNAs of multiple individuals of mutagenised population. In sample pools, heteroduplexes with a mismatched base pair are formed between wild-type and mutated DNA fragments by denaturing and re-annealing PCR products. Heteroduplexes are cleaved by an endonuclease enzyme able to recognise the mismatch position. Cleaved products are then resolved using denaturing polyacrylamide gel or capillary electrophoresis. When a positive signal is identified, individual DNA samples of a pool are separately analysed to identify an individual mutant plant and the induced mutations are eventually confirmed by sequencing. In Diploid species and gene-rich genome phenotypic alterations induced by mutagenesis are easy to identify because point mutations often occur in a functional region and they

are not masked by gene redundancy. This method together with comparative computational approaches favoured to analyse rare mutations with high sensitivity and specificity. Other approach used in mutation is EMAIL (Endonucleolytic Mutation Analysis by Internal Labelling) which is a mismatch scanning assay involving capillary electrophoresis and internal amplicon labelling by PCR incorporation of fluorescently labelled deoxynucleotides. Bajaj et al.[49] reported EcoTILLING-based large-scale allele mining and genotyping strategy implemented for association mapping is much effective for a diploid genome crop species like chickpea with narrow genetic base and low genetic polymorphism.[34]

Major outcomes and advancements

In pulses mutation breeding has contributed significantly. In chickpea, 21 mutant varieties developed through different mutagens at world level (http://mvgs.iaea.org). Maximum varieties have been developed through gamma rays. The Indian Agriculture Research Institute (IARI), New Delhi and the Nuclear Institute of Agriculture & Biology (NIAB), Faisalabad, Pakistan developed different disease resistant varieties of chickpea through mutation breeding. The Bangladesh Institute of Nuclear Agriculture (BINA) Mymen Singh worked for the improvement of nutritional quality of chickpea. The work on mutation started long back, but the first high yielding chickpea mutant cultivar 'CM-72' resistant to Ascochyta blight was released in Pakistan in 1983. In 1995, a new mutant cultivar 'CM-88' was released with multiple resistance (Ascochyta blight and Fusarium wilt) and recently 'CM-98', which is disease resistant and high yielding. The current area covered by these mutant cultivars is 350,000 ha, more than 30% of the total area under chickpea in Pakistan. The first Indian mutant variety 'Kiran' (RSG-2) was released in the year. In addition to release of different varieties several mutants have been used as parents in chickpea improvement program In India, four high yielding and Ascochyta blight and wilt disease resistant chickpea mutant varieties Pusa – 408 (Ajay), Pusa – 413 (Atul), Pusa – 417 (Girnar) and Pusa – 547, developed at I.A.R.I., New Delhi, and released by the Indian government for commercial cultivation, are the first examples of direct use of induced micro-mutants in a legume crop in the world. Beside high yield performance under late sown crop, chickpea mutant variety Pusa – 547, released in 2006 for farmers' cultivation, has attractive bold seeds, thin testa and good cooking quality. The Bangladesh Institute of Nuclear Agriculture developed chickpea cultivar Faridpur-1 (Hyprosola) through gamma rays which had 20% higher yield and 20% higher protein content than the parental cultivar Faridpur-7. Through mutation breeding one variety each released by Egypt and Turkey.[37,50-53] The mutant cultivars have contributed immensely in augmenting the efforts of Indian plant breeders in achieving the target of food self sufficiency and strong economic growth, the major breakthroughs are mentioned in Table 1.[61–72]

Table 1 Promising chickpea varieties developed through mutation breeding

Name	Parent variety	Year	Characters	Ref	Agencies
RSG-2 (Kiran)	RSG-10	1984	High pod number, early, high yielding with salinity tolerance	Dua et al.[54]	ARS Durgapura, Jaipur
Pusa- 408 (Ajay)	G-130	1985	Moderately resistant to Ascochyta blight	Kharkwal et al 1988,[37]Micke,[8] Kharkwal et al.[51]	I.A.R.I., New Delhi
Pusa-413 (Atul)	G-130	1985	Moderately resistant to Ascochyta blight	-do-	I.A.R.I., New Delhi
Pusa-417 (Girnar)	BG-203	1985	Resistant to wilt, moderately resistant to stunt and root rot	-do-	I.A.R.I., New Delhi
Pusa-547	BG-256	2006	Attractive bold seeds, thin testa and good cooking quality	Kharkwal et al.[53]	I.A.R.I., New Delhi
BGM-547	BG-256	2006	Tolerant to wilt, root rot and stunt diseases	-do-	I.A.R.I., New Delhi
CM-72	6153	1983	Resistant to Ascochyta blight	Haq et al.[55]	NIAB, Pakistan
CM-88	C-727	1995	Resistant to Ascochyta blight and Fusarium wilt	Haq et al.[56]	NIAB, Pakistan
CM-98	K-850	1998	Resistant to Ascochyta blight and wilt	Haq et al.[55]	NIAB, Pakistan
CM-2000 (Kabuli)	ILC-195	2000	Resistance to diseases	Haq et al.[57]	NIAB, Pakistan
CM-2008	Punjab-1	2008	Improved seed size, resistance to wilt	Shah et al.[44]	NIAB, Pakistan
Faridpur-1 (Hyprosola)	Faridpur-7	1981	High yield, earliness and high protein	Oram et al.[58]	National Seed Board of Bangladesh
Binasola-3	G-97	2001	Early maturity, erect plant type, larger seed size and rough seed coat	Shamsuzzaman et al.[59]	National Seed Board of Bangladesh
Line-3	NECL #055	1992	High yield	Moustafa,[60]	Egypt
TAEK-SAGEL	AK 71114	2006	Ascochyta blight resistance and better quality	Sagel et al.[29]	Variety Registration and Certification Centreş Ministry of Agriculture and Rural Affairs Turkey

Conclusion and prospects

Mutants are effectively used for studying gene expression, gene regulation and assigning functions to genes. Induced mutations are necessary to enhance rate of genetic variability, introducing multiple trait, identify trait specific genes in order to set up molecular gene database, study molecular functional genomics and develop bio-informatics for future. Isolation of mutants ideal to grow under climate change and resistant to abiotic and biotic stresses along with the high yield to combat low productivity of chickpea is the major aim. The developing countries with high population growth can't wait before genetic engineering can reap high harvest. The general strategy for reverse genetics called TILLING (Targeting Induced Local Lesions in Genomes) or coming together of traditional mutagenesis with functional genomics and EcoTILLING, a variation of this technique, represents a means to determine the extent of natural variation in selected genes in crops. Reverse genetic approach combines high frequency of point mutations induced by special mutation techniques can detect hetero duplexes between wild type and mutant DNA fragments using 'denaturing high performance liquid chromatography' or 'DHPLC'. In this approach point mutation of high density are required for which highly efficient chemical mutagens and ionizing radiations are generally used to develop of mutated generations.

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None.

Conflicts of interest

The authors declare that there is no conflict of interest.

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