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Mutagenesis in soybean: A review

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Abstract
It has always been a continous practice to search for technologies and advances to address the problem of food insecurity resulting due to the increasing population. Increasing the production of varieties oriented towards the ongoing demands as well as climate resilient varieties is the need of the hour. This literature highlights the applications of mutations in the improvement of Soybean. Mutation breeding has proved to be a important supplement along with other plant breeding techniques in creating new genetic variability, improving crop architecture, enhancing biochemical constitution as well as enhancing the growth and development cycles. The agents that create mutations are termed as mutagens. Both physical as well as chemical mutagens are used to induce mutation in seeds and planting material. After induction of mutation the mutant lines are selected on the basis of desirable traits in various field trials whereby the extreme mutants are discarded. Finally only the desirable mutant lines at multilocation trials are selected as a new variety or used as parents in hybridisation programme. Therefore the use of mutations as well as the different agents used to create mutations in particularly Soybean has been well documented and reviewed in the following literature.

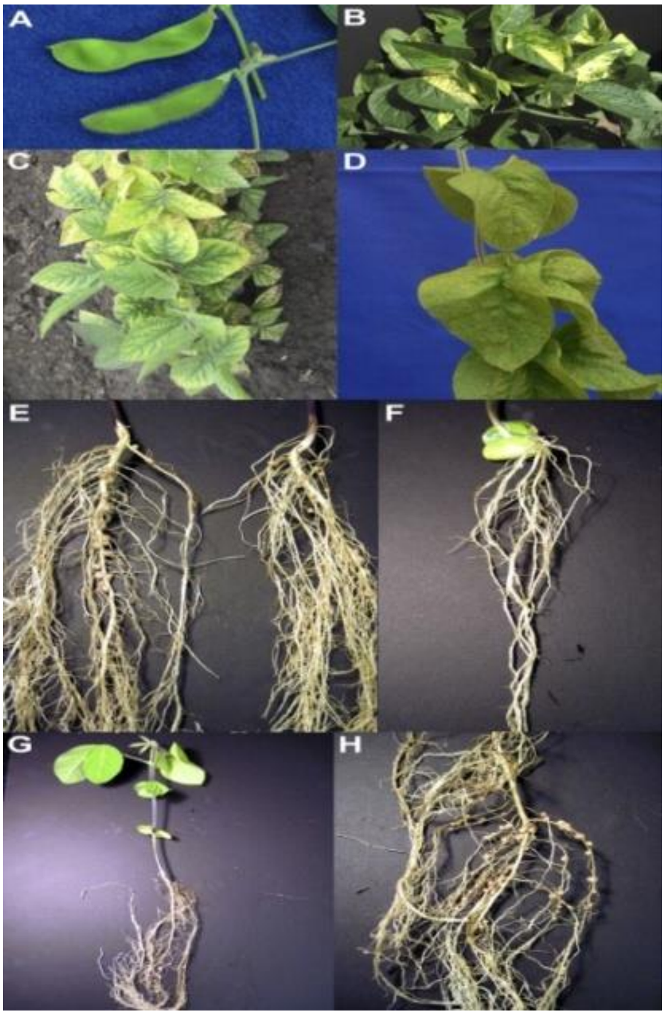
Keywords: Mutation breeding, genetic variability, biochemical constitution, multilocation trials

1. Introduction
Soybean [*Glycine max* (L.) Merrill] is considered as a miracle crop due to its triple usage as it has a high content of protein, a rich oil seed crop as well as a beneficial forage crop. Soybean contains 40% protein with high amount of amino acids, 20% oil that contains poly unsaturated fatty acids like omega- 6 and omega-3 fatty acids in abundance. This crop is also rich in minerals comprising of 6-7% total minerals, 8-10% crude fibre and 17-19% carbohydrates. Apart from these soybean is a rich source of micronutrients like iron, vitamin B-complex and isoflavones. These isoflavones are a major reason behind a lot of health benefits provided by soybean like prevention from cancer, recovery from diabetics and also major menopausal problems that occur in women. The use of plant breeding for crop improvement dates back to start of human civilization. Mutation breeding is termed as development of new varieties by utilizing the genetic variability created through physical or chemical mutagens. Mutation breeding has helped breeders to a great extent in creating novel traits as it becomes difficult with classical breeding techniques due to the inadequacy of variability in existing germplasm. Mutation breeding in soybean has been useful for developing varieties with enhanced biochemical traits like protein content, oil content, low phytate content etc. It also provides resistance to major stress both biotic as well as abiotic such as disease resistance, major weeds and insects. The following review includes the role of mutagenesis in the improvement of soybean.

2. Types of Mutation
Mutation as already defined is a sudden heritable change in any particular trait of an organism. These occur due to the changes in the base sequences of gene also known as point mutations. The term mutation was coined by ‘Hugo de Varies’ in the year 1990. Basically mutation is categorised into two main types spontaneous and induced mutations. Spontaneous mutations are those that occur naturally and whose frequency of occurrence is very low i.e. one in ten lacs where as on the other side induced mutation are those that are artificially created with the help of some physical and chemical agents known as ‘mutagens’. The phenomenon of utilizing induced mutation for crop improvement is known as mutation breeding Unlike spontaneous mutation, induced mutation have a much higher frequency which makes it relatively easier to work with them.

2.1 Physical and Chemical mutagens

The credit for discovering mutagens and utilizing them for the first time to produce variation goes to HJ Muller in 1928. Mutagens are of two types physical or chemical. Physical mutagens include Ionizing Radiation like UV light, X ray, Gamma rays & neutrons. Among these Gamma rays and X-rays are non particulate electromagnetic, ionizing radiation and neutrons are particulate ionizing radiation where as UV-rays are non ionizing radiation. The Chemical mutagens are categorized into 4 categories *viz.* Alkylating agents that includes EMS (Ethyl methane sulphonate), MMS (Methyl methane sulphonate), nitroso compounds, diazoalkanes etc. Acridine dyes that consists of acriflavine, proflavin, acridine orange, ethidium bromide. Base analogues these include 5-bromouracil & 5-chlorouracil and others include sodium azide, hydroxyl amine etc. Figure 1 shows the phenotypic variations observed in soybean plant treated with Fast neutrons. (Yung-Tsi Bolon *et al.*2011) [34].



***Source*:** Yung-Tsi Bolon *et al.* 2011

Fig 1A: Short trichome mutant(top) compared to wild type(bottom), B. Altered leaf pigmentation, C. petite yellow- tinged mutants, D. short petiole and curled leaves, E. roots after 3 weeks of germination, F. no nodulation and abnormal shoot root proportion, G. Elongated internodes, H. Hyper nodulation.

3. Applications of Mutagenesis in Soybean

3.1 Effect on Morphological-Biochemical traits and Cytogenetic variations

In a study conducted by Mendhulkar *et al.* (2015) [28] on seeds of [*Glycine max* (L.) Merrill] they treated healthy and uniform seeds with 0.02, 0.06 and 0.1% of EMS(ethyl methane sulphonate) for 4 and 6 hrs respectively. The fluctuations in mitotic index, variations in biochemical content and chromosomal aberrations as well as variations in morphological traits were observed. Out of all the treatments the one with 0.1% concentrations for 6 hrs proved to be the most adverse as it produced the highest level of chromosome abnormalities (9.96%). The mitotic index decreased significantly to half (6.77%) as compared to control. The biochemical variations include high protein content at high EMS concentration for longer duration of treatment. The variations in morphological traits were change in leaf margin, early flowering & chlorophyll chimeras. Some other chromosomal aberrations include unequal distribution of chromosomes, precocious movement and chromosomal laggards. In Figure 1,2 & 3 respectively the various morphological variants of this study are depicted clearly.

3.2 Effect on physiological and quantitative characters

The effect of mutagen Gamma rays was studied on physiological traits like seed germination and plant survival by Aditya *et al.* (2017) [2] on two varieties of Soybean BSS-2 and RKS-18. The seed germination recorded in both the varieties was found to be lower than the checks. It was seen that higher dose of mutagen in both the varieties was the reason behind the less germination percentage. However the germination percentage was higher in variety BSS-2 as compared to RKS-18 where as survival percentage was higher in seedlings of RKS-18 which revealed a higher genetic damage in the variety BSS-2. Effect of mutagen on quantitative traits like days to maturity, days to 50% flowering, number of branches per plant, number of seeds per pod, pod length plant height(cm), 100 seed weight(g), number of pods per plant, and yield per plant(g) was also recorded. Almost all mutagen doses except for some brought decrease in traits like plant height, number of branches per plant, pod length, number of seeds per pod and seed yield per plant in both the varieties BSS-2 and RKS-18. It was concluded that the reasons associated for this decrease is the genes responsible for these traits are distributed throughout the genome which may have been affected by mutagens that resulted in different kinds of micromutations.

3.3 Mutagenesis through Genome editing technology

Jianan Han *et al.* (2019) [16] conducted a study on soybean in Beijing where they used *Agrobacterium*-mediated transformation to introduce CRISPR/Cas9 expression vector in cultivar ‘Jack’. This lead to the creation of targeted mutants of E1 gene that is responsible for flowering in soybean. Two types of mutations were obtained, 11bp and 40 bp deletion at E1 coding region respectively and also frameshift mutations which lead to the production of premature translation termination codons and truncated E1 proteins, which causes early flowering under long day conditions. It was found that critical diminished E1 gene expressions of two novel mutants showed that the shortened E1 protein disinhibited GmFT2a/5a and expanding GmFT2a/5a gene expressions gave about clear early blossoming. Therefore in this study a very recent yet advanced technology *viz.* CRISPR/Cas9 was used along with the concept of mutation and *Agrobacterium* based transformation to introduce the cas 9 expression vector into soybean cultivar and knocking out of the E1 gene and comparing the effects of novel E1 mutants to flowering.

In another study conducted by Chenlong Li *et al.* [24] in 2019 genome editing technology CRISPR/Cas9 was used to detect mutant alleles of the two major storage proteins *viz*. Conglycinins and glycinins that constitute almost 70% of total soy seed protein and are encoded by a small family of genes. The entire study was designed to test sgRNAs that were targeted to nine storage protein genes out of which they detected DNA mutations in three storage proteins in soybean roots. These results will play a major role in creating a new and useful resource for breeders to engineer and produce varieties with such mutations.

3.4 Quality enhancement

Soybean oil comprises of 12% palmitic acid, 4% stearic acid, 23% oleic acid, 54% linoleic acid and 8% linolenic acid. Breeders have always focussed on creating soybean cultivars having less saturated fatty acids to enhance the oxidative stability and also to improve the quality of oil. This will happen by reducing linolenic acid and increasing oleic acid. Kim *et al.* (2015) [20] in their study aimed to characterize the low linolenic acid trait in seed oil of cultivar PE1690, this cultivar had undergone induced mutagenesis with the help of EMS. The examination of PE1690'S DNA revealed a point mutation that substituted guanine in place of adenine at the second intron splice site of the GmFAD3A gene. This mutation was responsible for missplicing this led to the inclusion of 334 bp of the second intron into the coding sequence. The amino acid chain of this mutant was analysed and this revealed that the position of 128 amino acid that codes for tryptophan (TCG) in PE1690 had changed to a premature stop codon. Therefore this single point mutation has turned the desaturase enzyme non-functional. The relationship between this PE1690 derived GmFAD3A allele with linolenic acid was concentrated with the help of a marker by testing in 89 F2 progeny of a cross made between Pungsannamul (wild type) × PE1690. The results proofed that the mutation was associated to low linolenic acid content in seed oil.

In another study conducted by Austin *et al.* (2017)[4] where the mutagen used was fast neutron. During the screening of the mutant population a different phenotype was identified which had almost twice the amount of sucrose and less than half of oil as compared to the wild phenotype. However using methods like genomic hybridisation, bulk segregant analysis and backcross breeding were used to find the relationship between the seed composition phenotype and reciprocal translocation in chromosomes 8 & 13. The translocation in a backcross population cosegregated with seed composition phenotype and followed non mendelian segregation patterns. It was hypothesized that the translocation was the cause behind the different seed composition. Still this study raises a huge scope for research and creates new research directions as this mutant can serve as a vital germplasm for further breeding programmes in soybean.

Phytic acid is the only stored form of phosphorous in plant tissues. During a study conducted by Sweta Kumari *et al.* (2014) [22] 17 genotypes of soybean were treated with chemical mutagen EMS and Physical mutagen gamma rays. This led to the development of 34 mutant populations. Screening of mutant populations M2-M10 was done on the basis of resistance against YMV. In M10 some plants showed reduction in phytic acid, protein and mineral content after complete analysis. These results gave us 5 mutant lines namely IR-JS-101-4, IR-V-101-3, IR-DS-118-2, IR-DS-119-4 & IR-DS-122-2 that had less amount of phytic acid as compared to wild type.

3.5 Biotic stress resistance

Soybean cyst nematode (SCN) is considered to be a devastating pathogen in the production of soybean. To combat the losses it is very important to identify more resistance sources to bring out novel genes from these genetic sources. With this aim in a study by Feng-Yong GE *et al.* (2018) [13] two EMS induced mutant populations (M_2) of PI 437654 showing a broad resistance to almost all SCN races and the other of Zhonghuang 13, a soybean variety from China showing resistance to lodging were produced. 13 mutants were identified after screening 400 mutants of the PI 437654 M_2 Population that showed alteration in resistance to race 4 of SCN. These 13 mutants were examined to see if they show changes in genomic sequences of the 3 known resistance genes compared to the wild phenotype and it revealed that all of them were still resistant to race 3 exactly like the wild phenotype of soybean. Therefore this study reveals that those 13 mutants carry a mutation of the genes that are responsible for the resistance to SCN race 4 in PI 437654.

Another devastating biotic stress that hinders the production of soybean is the incidence of diseases like Charcoal rot and Yellow vein mosaic (YMV). With respect to this a major milestone is the release of the variety AMS-1001 which is an induced mutant of the famous variety JS 93-05. (S.S. Nichal *et al.* 2020) [29]. This variety has higher yield as compared to state check MAUS-71 by 24.17% and national check JS-335 by 20.58%. It has also high oil content upto 23.90% compared to JS-335. It is highly resistant to diseases like charcoal rot and YMV along with having determinate growth habit, glabrous pods/stem and purple flower colour. This variety was released by SVRC (State variety release committee) under the name AMS-1001 (PDKV Yellow Gold) for the state of Maharashtra and national name of this variety is IC 626343.

3.6 *In- vitro* applications

Mutagenesis has also found wide applications in tissue culture technologies for producing mutant cultures. One such experiment conducted by Hafez *et al.* (2019) [17] in which healthy calli of soybean cultivar 'Giza 111' was produced and the impact of two chemical mutagens *viz*. sodium azide and ethidium bromide on its fatty acids levels. The culture was developed in Murashige and Skoog medium for 14 days, this gave rise to plantlets. Induced callus from hypocotyls (H), epicotyls (E), leaf (L) and cotyledon (C) were tried with different concentrations of 2,4-dichlorophenoxy acetic acid and N6- benzyladenine. Both H and E explants were found out to be the best for callus expansion. Development elements of H as well as E calli were set up. The critical harvesting period of the calli was the 4th week. H-calli came out to be the fast growing one. The H- calli was given the mutagenic treatment at concentration of 0.5, 1.0 and 1.5 mM of each mutagen with two drenching times (1/2 and 1 hr). After one month of mutagenic treatment the refined callus was examined for fatty acid profiling and compared with control. The fatty acid profiling was done using gas fluid chromatography. The Fw pattern of SAs and ETBr treated calli has lesser values than control but was quite similar. However it was seen that the content of saturated fatty acids and unsaturated fatty acids could be raised up by expanding the concentration and time of incubation.

3.7 Effect of mutagen on seed storability of Soybean

A study was conducted on three soybean genotypes *viz*. Gmx

92-6-10, Gmx 92-5-4^E and TGX 87^{D}1303 using ^{60}Co gamma irradiation at 0.50,100,150,250 or 300Gy by Addai & Safo-Kantanka, (2006) [1]. Large no. of seeds of all three varieties were treated with 250Gy (LD_{50}) and the variants from M_2 and M_3 generations were screened in two categories. The first category was of threshed seeds and the second category included the unthreshed seeds at a temperature of 22-25°C and humidity of 30-35% in laboratories for 4 months. After screening the seeds that showed germination above 80% were selected. The results revealed that highest no. of variants showing high storability in M_2 generation were from genotype Gmx 92-6-10 whereas in M_3 generation highest no. of genotypes were recorded from genotype TGX 87^D 1303. However, variability in threshed seeds was more than unthreshed seeds and therefore more no. of selection was carried out from threshed seeds.

4. Future Prospects

It is a well known fact that sunlight that is injested by the Earth's surface is reflected back as a long wave radiation. This leads to expansion of environmental convergences of CO_2 and other ozone harming gases that get trapped and increase heat leading to the worldwide problem of global warming. But in a study it was found that if we grow a chlorophyll deficient mutant of soybean in field the amount of solar radiation that is reflected rather than absorbed is higher as compared to commercially grown varieties. In any case, while the impact on radiative constraining during the harvest cycle at the size of the individual exploratory plot was discovered to be huge (−4.1± 0.6 W m−2), worldwide replacement of the current assortments with this genotype would cause a little expansion in worldwide surface albedo, bringing about a worldwide shortwave radiative compelling of −0.003 W m−2, comparing to 4.4 Gt CO2eq. The thought behind reduction in the surface-driven radiative constraing mediated through chlorophyll deficient mutant crop requires the availability of high yielding and high albino crops.

Table 1: List of Mutant varieties of soybean released since the year 2000

S. No.	Variety Name	Country	Year
1	Aomori-Toyomaru	Japan	2001
2	Akita-midori	Japan	2002
3	Chiang Mai 5	Thailand	2006
4	TAMS 38	India	2007
5	Beinong 103	China	2009
6	Albisoara	Maldova, Republic of	2010
7	Amelina	Moldova, Republic of	2010
8	Rosa	Bulgaria	2010
9	Gamasugen 2	Indonesia	2013
10	DT2010	Vietnam	2017
11	Kemuning 1	Indonesia	2019

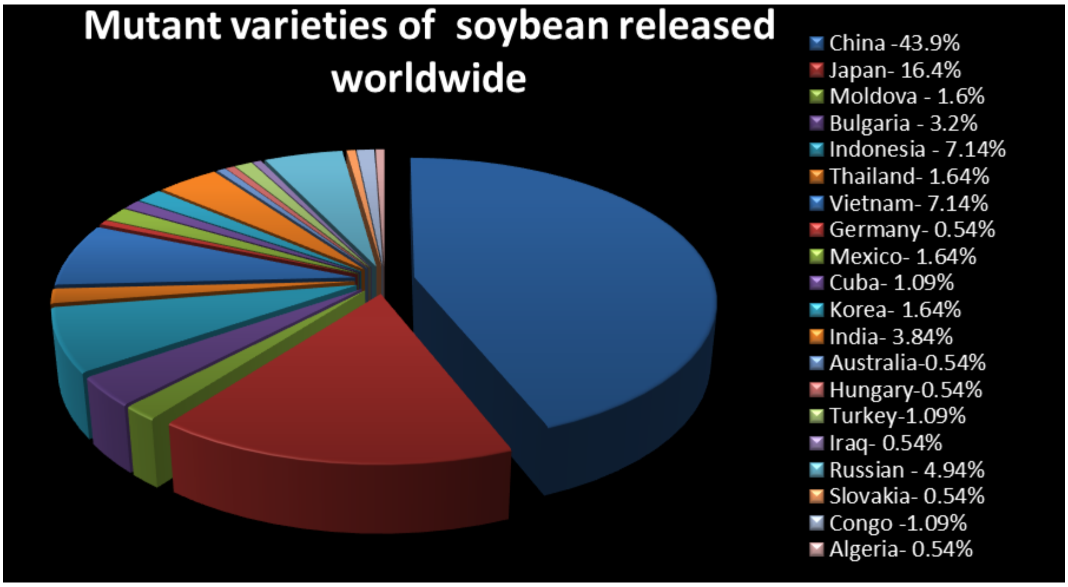


Fig 2: Pie chart showing contribution of countries in mutagenesis of soybean

5. Conclusion and Discussion

According to recent information from the International Atomic Energy Agency (IAEA) Mutant Variety Database ©2021 around 182 mutant varieties of soybean have been recorded. These mutations have been induced with the help of physical as well as chemical mutagen. Table 1 shows a list of soybean varieties developed since the year 2000 worldwide. It has been noticed that China contributes to the maximum mutant varieties of soybean around 43.9% whereas countries like Germany, Australia, Hungary, Iraq, Slovakia & Algeria contribute the least around 0.54% of the total varieties released. Figure 1 shows a graphical representation of the contribution of different countries in producing mutant varieties of Soybean. The different studies conducted on mutagenesis of Soybean concludes that mutation breeding has proved to be a great phenomenon for crop improvement. It is responsible for creating new germplasm resource which will help breeders in future generation for creating milestones in plant breeding. Mutagenesis can also be used with new recent advances in technology like CRISPR/Cas 9 as well as *in vitro* applications like tissue culture etc which suggests that it constitutes in an holistic approach using all technologies for crop improvement. With the help of mutagenesis it is easier to modify the genetic constitution of crops according to the desirable traits which also enhances selection of crops. Mutation breeding also breaks the barriers in production of soybean due to biotic and abiotic stresses as well as the studies also indicate the vast applications of mutation breeding in quality enhancement of soybean. Soybean being a commercial crop also proves to be an ideal part of human diet therefore it is very important for soybean to meet the demands of customers regarding its quality and that's why quality enhancement of soybean through mutagenesis is highly preferable. The applications of mutation breeding is vast not only in soybean but also in other crops.

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