Phyton-International Journal of Experimental Botany
DOI:10.32604/phyton.2020.013679
Review

Ethyl Methanesulfonate as Inductor of Somaclonal Variants in Different Crops

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Received: 17 August 2020; Accepted: 24 September 2020

Abstract: Ethyl methanesulfonate is a chemical mutagen, which is currently being used in plant breeding, to increase genetic variability in genes of agronomic interest, of species useful in agriculture. It primarily causes single base point mutations by inducing guanine alkylation, resulting in GC to AT transitions. Its effect is different between clones of a genotype and between genotypes of the same species. This review presents the results obtained in recent research, where its effect on plant tissues, callus, and cells in suspension has been evaluated. Changes in the phenotypic expression of somaclonal variants were reported, involving morphology, production of secondary metabolites, changes in metabolic routes of resistance, tolerance to stress, increased seed yield, among others. In addition, this review compiles the doses and guidelines to consider before using this mutagen, which can serve as a guide for future trials in deciding the response variables, the type of plant explants and the selection of the study model. Mutant lines have allowed plant breeders to have a collection of plants with different characteristics, in places where the cultivar does not have its center of origin. It is important to note that it is still necessary to continue evaluating the heritability of mutations and their behaviour in the environment where they will be established, in order to obtain new varieties of plants that can be cultivated with uniformity in their genetic response.

Keywords: Genetic variability; plant breeding; point mutation; guanine alkylation; chemical mutagen

1 Introduction

The discovery of the mutagenic effect of X-rays at the beginning of the 20th century aroused interest in inducing mutations, mainly in plants and microorganisms. For this purpose, Stadler in 1928, began the work of mutagenesis in plants, and obtained mutant lines of corn and barley using X-rays [1]. Auerbach [2] was one of the first to publish works on chemical mutagenesis using mustard gas. Subsequently, Ethyl methanesulfonate (EMS) was used in pollen to induce mutagenesis in maize and in the 70s, they studied the use of carriers in combination with chemical mutagen, with the aim of reducing the damage caused to pollen germ cells [3]. However, with the genetic reversal studies known as Induced Local Lesions in the Genomes (TILLING), chemical mutagenesis reappeared [4]. Chemical mutagens (MCs) are currently being used in plant breeding, the most widely used are the following: EMS, nitrosoethylurea, N-methyl-N-itrosourea,

colchicine, sodium azide and ethyleneimine. Ethyl Methanesulfonate is the most widely used for crop improvement, presenting 106 registered mutant varieties [5].

Recent research has shown that the use of EMS is more efficient and effective to induce mutations in plants of agronomic interest, compared to other chemical and physical mutagens. Point mutations were obtained when using chemical mutagens, an advantage over radiation that generates chromosomal deletions and aberrations [6].

Andorf et al. [3] identified that EMS increased the mutation frequency in the embryo of corn seeds by 300%, compared to the other treatments with gamma irradiation, sodium azide and N-ethyl-N-nitrosourea. Likeise, Asif et al. [7] evaluated the mutagenic effectiveness of EMS and gamma rays in seeds of *Nigella sativa* L. var. Azad Kalaunji-1, finding the highest values in the EMS treatment (0.1%), followed by gamma rays (50 Gy γ-rays), and then the combination of gamma rays + EMS (Gy γ-rays + 0.1%). These studies showed clearly that the EMS was more successful than gamma rays in terms of effectiveness and efficiency in *in vitro* mutagenesis, being the most powerful among the chemical mutagens used in rice [8].

Godfroy et al. [9] evaluated the effect of UV-C and EMS on a strain of brown algae Ectocarpus, they founded that the use of UV-C generated more mutations with respect to EMS, one of the possible reasons is the use of chemical mutagenesis protocols based on studies carried out in plants and the lack of knowledge of the effect of EMS in this species.

EMS is used to generate resistant and tolerant plants to biotic and abiotic stress, for this purpose, Shi et al. [10] found a mutant with a greater number of expressed genes that allowed it to generate mechanisms to tolerate low temperatures (down to –3°C). Other investigations have identified defence mechanisms against pathogens in different crops, including an increase in the activity of phenylalanine ammonium lyase (PAL) [11] and an increase in the content of secondary metabolites, mainly flavonoids [12] and proline [13].

Taking into account that EMS is currently the most widely used chemical mutagen for plant breeding and a review has not been reported where the main research findings on the use of this mutagen are mentioned, this review will delve into its use of EMS to increase of genetic variability, emphasizing the results obtained in cultivable plants.

2 Ethyl Methanesulfonate (EMS)

Colloquially known as EMS, its chemical formula is $CH_3SO_3C_2H_5$. It is a monofunctional alkylating agent, generally liquid, highly soluble in organic solvents, slightly soluble in water and its molecular weight is 124.2 [14].

2.1 Mechanisms of Action of EMS

The ethyl methanesulfonate (EMS) has been confirmed to cause primarily single base point mutations hundreds to thousands of heritable mutations can be induced in a single plant line [4]. Rajasekar [6] and Serrat et al. [15] reported that EMS induces guanine alkylation (Fig. 1), induces nucleotide substitution or base changes, which consequently alter codon sequences; due to this mispairing-alkylated G pairs with T instead of C, resulting in GC to AT transitions., these transitions occur due to the alkylation at the O^6 position of guanine.

EMS owes its biological reactivity to its ethyl group. The transfer of the group occurs via SN1 (substitution, nucleophilic, unimolecular) or an SN2 (substitution, nucleophilic, bimolecular) mechanism [16].

In the 70s it was reported that mutagens that react through the SN1 mechanism produce higher amounts of O^6-alkylguanine in relation to N^7-alkyiguanine, therefore, EMS, which can react through an SN1 and SN2 mechanism, is expected that produces relatively more O^6-ethylation than that found with other mutagens that react via an SN2 mechanism (example: Methyl methanesulfonate) [17].

This finding was observed in more than 15 plant species [4]. Moreover, Acanda et al. [18] found that these transitions caused multiple alleles within a gene, leading to multiple phenotypes. Point mutations occur in the exonic regions [19], with higher methylation rates in the 5' regions [20].

Aasim et al. [21] documented that depending on the dose of the mutagen it is negatively correlated with the damage caused in the DNA, it was identified that the damage is greater in the root cellular nuclei, compared to those present in the leaves.

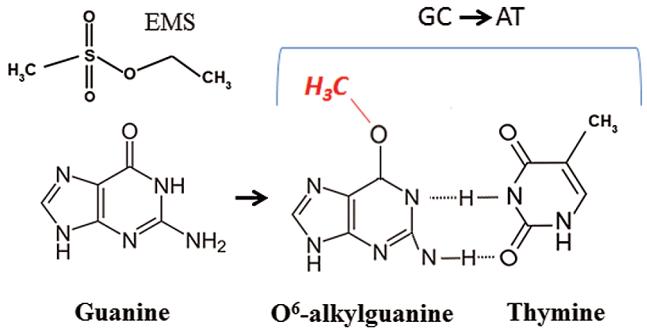

Figure 1: Alkylation of guanine and GC to AT transitions. (adaptation of: Yang et al. [20]; Marguison et al. [22])

2.2 Main Uses of EMS

On a commercial and scientific level, ethyl methanesulfonate is used as a mutagen. This compound has been used mainly in plant breeding, by putting it in direct contact with seeds, *in vitro* cultures and plant tissues. As mentioned by Chen et al. [23] and Lu et al. [19] *EMS* has been used to generate a collection of mutants, which allow having different variants in genes of interest. This is a useful tool for understanding gene function and determining the inheritance of mutations [3,23].

One of the challenges of plant breeding in agricultural is to generate varieties resistant to different types of biotic and abiotic stress, mainly diseases that limit food production, where agronomic practices, especially the use of synthetic fungicides have not had satisfactory results in some cultivars, due to anthropogenic and climatic factors.

Regarding the use of EMS to generate plants resistant to phytopathogens, Sánchez-Martín et al. [24] used mutants originated by the action of EMS to identify genes for resistance to various phytopathogens. In addition, Reyes-Zambrano et al. [11] evaluated *Agave americana* L and identified that EMS caused phenotypic modifications in the morphological and morphometric parameters, in addition to an increase in the activity of the enzyme (PAL), which is related to resistance to biotic stress.

The use of EMS has been also documented to generate plants resistant to abiotic stress. Bolívar-González et al. [25] carried out an *in vitro* evaluation of EMS on *Coffea arabica* L plants, in the state of cells in suspension; they detected somaclonal variants tolerant to increasing concentrations of salt.

The ethyl methanesulfonate (EMS) has been also used to increase the agronomic efficiency of plants of commercial interest, in particular to increase the production of seeds and secondary metabolites of interest [7]; in perennial fruit trees, EMS has been used to find dwarf plants under field conditions, after *in vitro* mutagenesis [12].

Godfroy et al. [9] performed comparative investigations between chemical and physical mutagens. They reported that EMS and X-rays generated mutations in a cell line. Banjare et al. [26] mentioned that chemical mutagenesis with EMS is the most effective and efficient method to generate new agronomic traits useful in the improvement of cultivable plants and to study the function of many genes and the phenomena related to metabolic pathways.

3 Phenotypic Responses Caused by EMS

In the Tabs. 1 and 2 observed the most representative phenotypic traits after mutagenesis, involve morphometric and delta changes in the biosynthesis of hormones and secondary metabolites, which can be taken into account as study variables for future research.

Table 1: Effect of EMS on plant morphology

Leaves	Flowers	Fruits	Seeds
· Different colorations · Abnormal morphology · Presence of trichomes · Different forms of leaves · Number of stomatas and foliar area decreased	· Changes in the fertility · Does not produce flowers · Smaller flowers · Rudimentary anthers · Different colorations	· Different colorations · Different forms · Seedless fruits	· Decreased fertility in some species · Large and tiny seeds · Different colorations · Different forms · Variation in testa texture

3.1 Dwarfism in Plants

Studies have shown that at high concentrations of EMS damages the biosynthesis of gibberellins (GA) and abscisic acid (ABA); mutagenized plants with dwarfism showed the lowest values of these hormones in the leaves that generated changes in physiological processes, such as lengthening of shoots and increased distance between nodes; otherwise with the activity of the enzyme peroxidase, which increases in the mutagenic population of mango (*Mangifera indica* L.) cv. Arka Puneet, compared to plants without EMS, suggesting that the enzyme inhibits growth elongation [12].

Mallick et al. [27] evaluated EMS in mandarin plants (two years old). They reported that EMS decreased the density of foliar tissue and the number of stomata, causing a reduction in plant height. Rime et al. [12] found that dwarf mutant plants due to the effect of EMS, showed a decrease in the number of stomata (width and length), and an increase in the contents of phenols and chlorophyll in the leaves.

Table 2: Effect of EMS on the production of metabolites in plant species

Plant		Increase	Decreased
Mangifera indica	[12]	· Phenols, Flavonoids, enzyme peroxidase and abscisic acid	· Gibberellins and chlorophyll
Nigella sativa	[7]	· Thymoquinone and gibberellins	· Not documented
Capsicum frutescens	[28]	· Capsaisin in red fruits · Carotenoids in the leaves	· Capsaicin in green fruits · Carotenoids in the fruits
Zea maiz	[29]	· Not documented	· Phytic acid
Agave americana	[11]	· Fructans and anthocyanins · Phenylalanine ammonia lyase	· Nitrogen content · Photosynthetic efficiency
Centrapalus pauciflorus	[30]	· Vernolic acid · Palmitic acid · Oleic acid	· Linoleic acid · Stearic acid

3.2 Leaf Morphology

Asif et al. [7] found a considerable number of variations in the leaves of *Nigella sativa* L. postmutagenesis, these included different colorations of the leaves (green, yellow and ivory tones), morphometric changes of the petioles, presence of trichomes, different forms of the leaf (bilobular and trilobed) and fold upwards, some plants showed abnormal foliar morphology.

3.3 Floral Morphology and Flowering

Ethyl methanesulfonate (EMS) mainly generates changes in the fertility of the flowers of the mutants. Some of them, plants do not produce flowers. The main variants, however, are related to the shape and coloration of floral organs, especially from white to pink or yellow; mutants have smaller flowers with rudimentary anthers, these variations can be attributed to loss of gene functions or due to induced mutations in genes involved in flowering pathways [7].

Concentrations of 5, 10, 20 and 30 mM of EMS induced plants with late and early flowering of two genotypes of *Cajanus cajan* L. Millspaugh. The reduction in flowering time is related to an increase in the production of gibberellins and flowering late with a decrease in the production of this phytohormone [31].

3.4 Morphology and Physiological Processes in Fruits and Seeds

Chen et al. [23] evaluated the incidence of EMS on Cucurbit seeds. They observed variation in the color of the fruit shell (light yellow, light green, yellow green), shape (oblong, oval) and the total number of fruits. This was attributed to the mutations induced in the floral organs, including both sexual and morphometric expression, which in turn affected the pollination processes.

Various investigations have documented a negative relationship between seed germination and concentrations of EMS [6,23,32–34]. Therefore, it is recommended to perform a dose-response analysis to determine the average lethal dose for the study model in particular. This process should also be performed for scarification (mainly in perennials) to calculate the imbibition concentration and the contact timeof EMS In this respect, Joya-Dávila et al. [35] mentioned that when soaking corn seeds in distilled water, they found imbibition process at 16 hours. At 24 hours the germination was negatively affected. When exposing seeds of *Azadirachta indica* and *Zingiber officinalea* in concentrations greater than 50% for 18 hours, the germination process was inhibited; in contrast, concentrations less than 50% increased the germination percentage and favored phenotypic expression, providing greater establishment vigor, root volume and foliar growth.

The Food and Agriculture Organization of the United Nations (FAO) [5] mentions that a proportion of plants from mutagenesis with EMS show decreased fertility. In rice, two contrasting results were reported; Nogoy et al. [14] found that rice seeds treated with EMS had a decrease in seed fertility and problems in the accumulation of photoassimilates, resulting in vain seeds. In contrast, Serrat et al. [15] treated rice plant calli with EMS and obtained fertile plants that prolonged their vegetative stage with respect to untreated calli, a characteristic that can increase seed yield. Results showing a relationship between the type of explant subjected to mutagenesis with the fertility of the plants obtained. The use of undifferentiated cells such as callus or cells in suspension helps to reduce the percentage of sterile mutants.

Asif et al. [7] found mutants of *N. sativa* with large and tiny seeds, the seeds of mutant plants of equal or smaller size, with respect to the control plants, showed different colors (white, black, bicolor and yellow), whereas the large seeds kept their black color. They also showed variability in shape (oblong, round). In *Phaseolus vulgaris* L. Mahamune et al. [36] reported important changes in the seed, mainly in the testa (black and white coloration) intense gloss and smooth or rough texture.

3.5 Biochemical Characteristics of Interest

The ethyl methanesulfonate (EMS) has been identified as being involved in the chlorophyll metabolic pathway, as demonstrated by Pawar et al. [37], who found three types of response in the color of the leaves, the most frequent was the uniform yellow color, followed by uniform yellow-green color and albino leaves. They concluded that the mutagenic effectiveness decreased with the increase in the dose of EMS or gamma rays. Interestingly, EMS provided higher numbers of chlorophyll mutants than gamma rays. Conversely, Arumingtyas et al. [28] mentioned that *Capsicum frutescens* did not show an effect on the chlorophyll content when treated with EMS, where the highest chlorophyll content was recorded in the leaves and in the young fruits, without variation with respect to the non-mutagenized plants. It has also been shown that EMS caused variation in the capsaicin content. In clones and genotypes of *Capsicum frutescenses* L., a positive relationship was reported between the dose of EMS and the content of the antioxidant in genotypes one and two, contrary to what was observed in genotype three. Overall, a tendency to decrease the capsaicin content with increasing EMS doses was observed. The authors mentioned that their accumulation comprised different phenological stages in the fruits, in genotypes one and two the ripe red fruits presented the highest values, however, in genotype three the accumulation was presented in young green fruits [28].

Other works have reported that EMS treatment modifies the carotenoid content, as it showed by Arumingtyas et al. [28], who evaluated concentrations of EMS on *C. frutescens* and reported that the mutants had higher concentrations of carotenoids in the leaves than in the fruits; They mentioned that EMS caused a specific mutation in the phytoene synthase gene (Psy1), which directs the metabolic flow towards the synthesis of carotenoids. Carotenoid synthesis involves the transformation of geranyl-geranyl diphosphate (GGDP) into phytoene by the action of the enzyme Psy1, this EMS mutation positively modified the characteristics of the fruit in the form of β-carotene, oleoresin and capsanthin.

In soy seeds, EMS treatment generated the suppression of the Myo-inositol phosphate synthase (MIPS) gene of soy (GmMIPS1), leading to the abortion of immature seeds. The characterization of this mutant indicated that MIPS genes are important in the early stages of embryo development [38], since by suppressing the MIPS enzyme, the myoinositol pathway and phytic acid formation were completely stopped; these biochemical pathways are involved in germination [29].

Abid et al. [29] identified that phytic acid is the main form of phosphorus storage, in many plant tissues, especially in seeds, on the other hand, non-ruminant animals lack the digestive enzyme phytase, necessary to separate phosphorus from phytate molecule; For this purpose, these researchers determined that the bioavailability of phosphorus from phytate can be increased by supplementing the diet with the enzyme phytase or by using mutants with low phytic acid content; As they demonstrated with the maize mutant-EMS obtained in the F2 generation, they reported a genetic reduction of phytic acid in the seed of up to 70%.

The effect of EMS has been also documented on the production of secondary metabolites of interest. Reyes-Zambrano et al. [11] found that the calli of *A. americana* L. treated with EMS showed a higher concentration of fructans and anthocyanins, compared to the untreated ones. Asif et al. [7] quantified the content of thymoquinone in the mutant line VLY3 of *N. sativa*. They found that this pharmacologically active compound increased by 21%. Aasim et al. [21] identified a decrease in the content of bacoside A, by increasing the exposure time of EMS in cells of *Bacopa monnieri* L. root explants. In leaf explants, regardless of the exposure time, the concentration of bacoside “A” was stable when treated with EMS. Rime et al. [12] found a positive correlation between the concentration of EMS and the content of phenols (increase of 53%) and flavonoids (increase of 90%), but up to the concentration of 0.8%, higher doses of EMS decreased the content of these metabolites.

Hadebe et al. [30] observed that treatment with EMS did not modify the oil content in the seeds of *Centrapalus pauciflorus*, contrary to what was found by Savant et al. [39], who observed in *Sesamum indicum* that of the treatment with EMS increased the oil content and the fatty acid profile.

4 Genetic Variability Induced by EMS

The first study on the EMS treatment of *Coffea arabica* seeds was carried out by Vargas [32], with the aim of inducing genetic variability in the species to improve the agronomic characteristics of coffee plantations in Costa Rica, by the induction of resistance to abiotic (salinity) and biotic (*Hemileia vastatrix*) stress. Lu et al. [19] identified 180 mutations, they indicated multiple mutations in the entire genome, with this finding, they will allow to identify the domain and functionality of the genes in maize and provide allelic variants of agronomic interest.

Abady et al. [40] evaluated the genetic improvement of *Arachis hypogea* through mutations to induce traits of agronomic interest, using EMS. They concluded that it is a low-cost technique that does not require special facilities. This technique has generated to date 3400 mutants that are being used for the genetic improvement of *A. hypogaea*.

This is important to mention that EMS has been used successfully in increasing the genetic variability of many of the cultivable species, mainly cereals and Solanaceae, in the phenotypic expression in the different phenological stages, first of all in vegetative growth and in the metabolic pathways involved in flowering and seed production [28].

5 Median Lethal Dose (LD50) of EMS Reported for Various Species

Determination of LD50 is the first step to initiate the induction of mutations. Below are the results obtained for LD50, in seeds, plant tissues and cell cultures (Tab. 3). These doses are adjusted to a specific time and concentration of EMS, which can serve as a starting point for future research depending on the genotype to be used, but can also change as the concentration decreases and the contact time increases. According to the type of explant to be used, as mentioned above for seeds, calculate the imbibition rate, however, in tissues and *in vitro* cultures the cell permeability. The above in order to reduce the amount of EMS to be used, thereby reducing the amount of mutagen as waste and reducing operating costs.

5.1 Vegetable Tissues

For this purpose, Pawar et al. [37] they evaluated the effect of EMS on rhizomes of *Zingiber officinale*, different doses and two immersion times at 0.15% and 0.30% for four hours, the doses were the variable where the highest percentage of mutations occurred.

It was also evaluated in *Dendranthema grandiflora* Tzvelev cv. Jaya, specifically, on the survival of the treated cuttings, it was determined that the LD50 for physical and chemical mutagenesis works is 21.47 Gy for gamma radiation and 0.22% for EMS [6]. Other researchers have worked with mango (*Mangifera indica*) cv Arka, such as, Rime et al. [12] they recommend using 0.8% EMS (exposure time is not mentioned) to induce genetic change in plant height.

In another research work, sections of *Allium sativum* of three genotypes were soaked with increasing concentrations of EMS for 10 days, using 40 teeth for each treatment and after that they were sown directly in the field, the percentage of germination and survival was quantified. The results obtained were that EMS increased the germination percentage in the three treated genotypes, with respect to the control, up to the concentration of 1.2%, higher concentrations generated a decrease; Contrary to the above, survival decreased in the IG-2010-3-2 and IG-2009-11-1 genotypes, however, in the Agrifound White genotype, at a concentration of 0.1%, it presented greater survival (80%) with respect to control (57.5%). The authors recommend as LD50 a concentration of 0.1% in the genotype IG-2010-3-2 and Agrifound White, with respect to IG-2009-11-1 doses of 0.4% [23].

5.2 Seeds

In this sense, Yashoda et al. [31] they evaluated two genotypes of *Cajanus cajan* L. Millspaugh, for six hours, subjected seeds to different concentrations of EMS, concluding that according to the response observed regarding the increase in yield and the decrease in harvest times, the LD50 is different for each one, for the BSMR736 genotype it is 30 mM and for ICPL87 it is 20 mM, confirming the importance of determining the LD50 for each species to be used as a study model.

Arisha et al. [41], they performed a death curve to determine the LD50 of *Capsicum annuum* L., having as response variable the germination percentage and the survival rate, the identified dose was 0.6% of EMS for 12 hours; For this purpose, they soaked seeds in distilled water for six hours at 20°C with orbital shaking (100 rpm), then they were treated with the mentioned dose of EMS.

Under this context, Kashid et al. [42] reported that for *Cicer arietinum* L. seed treatments with EMS the LD50 is 0.15% for two hours, the response variable was the yield and seed weight.

On the other hand, Vargas-Segura et al. [34] they mention that the mean lethal dose of EMS, for treatments with *C. arabica* seeds is between 160 mM to 240 mM for eight hours, in this range is where there are variations in terms of the percentage of germination and emergence, as in the length of the aerial part and the root; at lower concentrations it does not generate visible phenotypic changes and high doses generated embryo death.

Likewise, the combined LD50 (gamma rays 200 Gy + 0.3% EMS) has been evaluated on seeds of *Macrotyloma uniflorum* (Lam) Verdc for four hours, for which they demonstrated an increase in the yield of the seeds, in this way they demonstrated the effectiveness to induce variability with a method combining physical and chemical mutagens [43].

Table 3: Median Lethal Dose (LD50) of EMS reported for various species

Plant species		Type of explant	Dose/time	Result
Zingiber officinale	[37]	Rhizomes	0.15% and 0.30%/4 hours	· Higher percentage of mutations
Allium sativum	[23]	Garlic cloves	1.2%/10 days	· Survival decreased
Cajanus cajan	[31]	Seeds	30 mM 6 hours	· Increase in yield · Decrease in harvest times
Cicer arietinum	[42]		0.15%/2 hours	· Yield and seed weight
C. arabica	[34]		160 mM to 240 mM/ 8 hours	· Variation: Length of the root, germination and emergence
C. arabica	[25]	Cells in Suspension	185.2 mM/2 hours	· Salinity tolerant mutants
Glycine max	[44]		30 mM/4 hours	· Polymorphisms
Oryza sativa	[15]	Corns	0.2%/2 hours	· Increase the mutation density
A. americana	[11]		15 mM/2 hours	· Higher fructan content

5.3 Cells in Suspension

Hofmann et al. [44], they determined that the LD50 of EMS in suspension cultures of *Glycine max* L. cv. Iroquois (age: Six months), was 30 mM for four hours, the response variable was cell survival, the dose at which the highest number of polymorphisms was found. In this same sense, Xu et al. [45], they allude that in the interaction of EMS with suspended cells of *Musa spp*., The LD50 is 0.2% of EMS for 18 minutes, but

when they are used for 10 minutes, the regeneration capacity of the plant is greater in In comparison with untreated cells, it was also observed that as the exposure time increased (without changing the concentration) it decreased.

On the other hand, it is recommended to use 185.2 mM of EMS for two hours on cells in suspension of *C. arabica* L. var. Catuaí (200 mg of one month of culture), when it is desired to find mutants tolerant to salinity and with response variable the survival percentage [25].

5.4 Callus

Serrat et al. [15], they evaluated the interaction of EMS on rice callus, for which they determined that compared to a dose of 0.2% EMS for two hours it was efficient to increase the mutation density, however, Chakravarti et al. [46] they obtained the same results when evaluating more hours of exposure, which comprised six hours with the same concentration of EMS.

Another research work determined the LD50 on calli of *A. americana* L. at a concentration of 15 mM with a contact time of two hours, since it allowed the highest content of fructans [11].

According to the aforementioned, callus mutagenesis is more effective than seed mutagenesis, since embryogenic cells located on the surface of embryogenic callus are more exposed to the effects of chemical mutagen (EMS) than those of the complex and fully formed zygotic embryos [15].

6 Changes Caused in Agronomic Variables

According to Yashoda et al. [31], the plants generated by EMS, increased the grain yield per plant and weight of 100 seeds of *Cajanus cajan* and *Cypripedium arietinum* L. In this sense, Kashid et al. [42] reported an increase in the weight of 100 seeds with higher concentrations of EMS evaluated in two cultivars. so too, Awais et al. [8] they evaluated the effect of EMS on rice plants, they observed fluctuating results in terms of agronomic variables, related to yield, which were dependent on the genotype used.

Nogoy et al. [14], when working on mutant rice lines they found that the concentration of amino acids was affected during grain filling, increasing the tryptophan content by 20 times, in quality tests, the flavor of the grains after cooking it was similar to that found in wild lines.

In livestock feeding, the forages used are sought to have high protein content, but low lignin. The plants most used for this are corn and sorghum. According to the aforementioned, Bettgenhaeuser et al. [47] found EMS mutants of these species with low lignin content, compared to the control without EMS, which increases the digestibility of ruminants; This reduction in lignification is accompanied by the phenotypic expression of the midrib of the brown leaf, in addition, they mention that these mutant lines help to understand the pathways that control lignification.

6.1 Stress-Tolerant and Stress-Resistant Plants

Ge et al. [48], mention that after the induction of mutagenesis with EMS, the first step is to select plants that are tolerant and/or resistant to different types of stress, both those caused by phytopathogens (biotic) and those caused by agri-environmental factors (abiotic).

Various research works mention that the EMS mutagen increases the activity of the enzyme phenylalanine ammonium lyase (PAL), which is important in wound recovery and as a defence mechanism against infection by pathogens, this finding was corroborated when treating calluses of *Agave americana* with EMS, the researchers demonstrated that PAL activity increased by 80% [11], results similar to those found by Soleht et al. [49], where they observed that the PAL enzyme increased its activity in the rice cultivars Pari Ireng (72%) and Melik (66%) after infection by *Xanthomonas oryzae* pv. Oryzae.

Another research work showed that mutagenized *Psoralea corylifolia* plants increased the proline content, gradually with the increase in the EMS dose, going from 14.7% to 79.2% before flowering, a

case similar to that found in the activity of the ascorbate peroxidase (APX) that increased from 16% to 161% at the beginning of flowering and gradually decreased after flowering; The increase in proline, isoflavonoid and APX concentrations is an adaptive response to stress generated by EMS [13]. It is also important to mention that the APX enzyme is immersed in the protection of oxidative damage caused by H_2O_2, so, there is a positive correlation between isoflavone and reactive oxygen species (ROS) during EMS treatment, its production increases with increasing concentrations of EMS, also mention that flavonoids act as ROS inhibitors. The inhibition of reactive oxygen species as a means of intracellular signaling pathways is related in one way or another to the antioxidant expression of genes [50].

Bhat et al. [13], they evaluated the responses to stress in *Psoralea corylifolia* (Babchi) induced by EMS, the results mentioned that the accumulation of psoralens in the stages before and after flowering, was crucial to maintain the high antioxidant potential and biophysiological processes, including the transcription of several genes and the proper functioning of numerous signaling pathways that improve cellular defenses, in addition, they found the presence of other stress proteins/enzymes, including glutathione-dependent dehydrogenase, glutathione transferase, universal stress protein (spot K) and superoxide dismutase, unlike the control plants it was observed that these proteins were negatively regulated. An increase in the dose of mutagen also increased the content of these proteins, possibly caused by high concentrations of ROS.

Chen et al. [51], they evaluated the effect of the mutagenic EMS in *Begonia* × *hiemalis* Fotsch on the degree of resistance to *Rhizoctonia solani* Kuhnusando, it was demonstrated that the majority of mutagenized plants were susceptible to the pathogen, but some showed resistance; these resistant plants were morphologically different from the control plants.

On the other hand, Carneiro in 1983 was the first to try to induce resistance to *Hemileia vastatrix* in Brazilian coffee plants using seeds of *C. arabica* cv. Catuai and five concentrations of EMS for 54 hours [52].

Climatic changes such as the La Niña phenomenon (present in Colombia), loss of soil structure and poor agronomic habits are the main causes of the flooding of arable land. When a plant is stressed by excess water, photosynthesis and oxygen availability are considerably reduced, slowing down the accumulation of energy and releasing energy to counteract the efforts of the flood [53]. According to the aforementioned Avivi et al. [54] they evaluated 21 EMS mutants, in order to determine the resistance to flooding, of which mutants one, three and six presented phenotypic changes that allowed them to have high yields under flood conditions, these changes with respect to the control They were related to dwarfism, decrease in stem diameter, increase in the number of tillers, brix degrees and sucrose.

Thus, it was also demonstrated that EMS applications on cells in suspension of *C. arabica* variety Catuaí, allowed to identify a change in the amino acid profile in response to osmotic stress, caused by increasing concentrations of NaCl [25]. In agricultural production, when it increases the electrical conductivity in the soil, in hydroponics or in different substrates due to an excess of salts, it causes problems in cultivated plants. Therefore, Mishra et al. [55], mention that the main effect of salinity stress is the increase in the osmolarity of the soil solution that prevents the flow of water into the plant, in addition, it causes cellular damage by accumulation of salt ions, peroxidation lipids and ROS.

7 Evaluation of Mutation with EMS in Later Generations

Alamin et al. [56] they cultivated SFL1 rice plants, from the F2 generation of cultivar Zhenong 34, under the mutagenic effect of EMS, the results demonstrated phenotypic expression of the screw-shaped flag leaf. Likewise, the behavior of photosynthesis, chlorophyll and cellulose content, with respect to wild plants, was evaluated; observing significant changes in the mutant plants, in addition, Mendelian segregation was presented, with important changes in photosynthesis and chlorophyll content that were not present in wild plants.

Some negative effects have been reported when using EMS in seeds in later generations. According to the aforementioned, Arisha et al. [41] they worked with 2000 seeds of *Capsicum annuum* cv. B12,

mutagenized with EMS, in the F1 generation only a phenotypic mutation was observed, this mutant expressed a different growth habit and various leaf forms compared to the control without EMS, however, the effects of EMS were observed with greater intensity in the F2 generation, where the germination percentage decreased and a defect appeared in the chlorophyll metabolic pathway. These changes are attributed to the effect of EMS on the F0 seeds, which affected the embryo. Changes in the F2 generation appeared during the seedling stage in chlorophyll formation, causing slow growth in all phenological stages [34].

8 EMS and Plant Omics

The omic sciences have made it possible to identify the structure and function of the genes present in the gene pool of a cultivated species, reveal the expression of genes of agronomic interest in the face of certain inducing events and show genetic variations between individuals with different phenotypes [57]. The mutations induced in the genome by EMS have been successfully evaluated and characterized using methods based on polymerase chain reaction (PCR) and sequencing techniques. Several molecular markers have been used to characterize the genetic variation induced among them, restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and nucleotide polymorphism unique (SNP) [25]. RAPD markers have been used to assess the genetic variability induced by physical and chemical mutagens in several cultivable species [44,58–62].

Molecular analysis of *C. arabica* plants obtained by EMS, using AFLP, resulted in the presence or absence of DNA fragments (relative to control plants); the combination ACA * CAA was the one with the highest percentage of polymorphisms (6%) [32]. Bolivar-Gonzales et al. [25], used RAPD-PCR to detect possible genetic polymorphisms in embryogenic cultures of *C. arabica* exposed to EMS, by comparing the bands present or absent in the mutagenized genotypes, with the pattern of bands exhibited by the DNA of the control plants (without exposure to EMS), allowed the observation of 50 fragments, of which 11 (22%) exhibited polymorphisms, while the remaining 39 (78%) presented the same pattern of bands for all the samples analyzed. Perera et al. [63], evaluated 21 Inter Simple Sequence Repeats markers (ISSRs), in mutant plants of *Miscanthus giganteus* generated by exposure to EMS, they reported that three ISSRs markers produced a total of 28 fragments, of these, 12 (42.85%) were polymorphic. On the other hand, second generation sequencing has provided a platform to reveal base alterations that occur throughout the genome due to mutagenesis [64]. Mohd-Yusoff et al. [65], using second-generation sequencing, scanned two individually mutated third-generation descendants of *Lotus japonicus* (M3, designated AM and AS), to identify single nucleotide polymorphisms (SNPs) and reveal the effects of EMS on nucleotide sequences in these mutant genomes; M3, called AM and AS), to identify single nucleotide polymorphisms (SNPs) and reveal the effects of EMS on sequences of (nucleotides in these mutant genomes; the results obtained show an overview of the point mutations that occurred in the mutant genome, they also revealed how efficiently EMS mutates genomic sequences in a single randomly mutagenized plant throughout the genome.

Xiao et al. [66] analyzed the complete genome sequences of four EMS-induced *Solanum melongena* L. mutants, in total 173.01 GB of paired end reads were obtained, identifying 1,076,010 SNPs; The analysis via KEGG (Kyoto Encyclopedia of Genes and Genomes) showed 11 genes in the L6-5 mutant, involved in the biosynthesis of anthocyanins or biosynthesis of flavone and flavonol, the results of real-time PCR, showed that only the expression of the Sme2.5_06210.1_g00004.1 gene, named as UFGT (Flavonoid galactosidase transferase), decreased significantly in the L6-5 mutant compared to the WT mutant. Furthermore, they report that the expression of the Sme2.5_06210 gene.1_g00004.1 was lower in colorless eggplant compared to colorful eggplant in wild-type eggplant cultivar, suggesting that this gene may play a key role in anthocyanin synthesis in *S. melongena*. nucleotides in these (mutant genomes; the obtained results show an overview of the point mutations that occurred in the mutant genome, they also revealed how

efficiently EMS mutates genomic sequences in a single randomly mutagenized plant throughout the genome M3, called AM and AS), to identify single nucleotide polymorphisms (SNPs) and reveal the effects of EMS on mutant sequences.

9 Perspectives of Use of Ethyl Methanesulfonate in Plants

EMS proves to be efficient to increase the phenotypic expression of plants, among them, the size of the leaf and its content of secondary metabolites; Future research hopes to find new metabolites generated by mutant plants, which serve as useful raw material in medicine, as reported by Purente et al. [67], in *Chrysanthemum indicum* var. Aromaticum. Future investigations using EMS in triploid plants, with sterile natural seed, (with low genetic diversity), may increase genetic variability and phenotypic response, as reported by Perera et al. [63], with *Miscanthus giganteus*, a cultivar of commercial importance, of which research is being carried out on its biomass for the production of liquid fuel. Several authors mention that EMS generates some abnormal plants within a mutagenized population [7,12,20]. Mohd-Yusoff et al. [65], document that in *L. japonicus* mutants, in future research they hope to find the gene (mutated) responsible for the abnormal phenotype of the fruit color, which allows identifying which metabolic pathway is interrupted in the mutation with EMS They also propose that the effect of SNPs in the coding and non-coding regions could be manipulated to identify a causal of a phenotype of interest. EMS can help identify the functioning of genes implicit in specific metabolic pathways as documented by Abid et al. [29] in soybean EMS mutants, which after mutagenesis did not present the Myo-inositol phosphate synthase gene (GmMIPS1), causing abortion, immature seeds; the characterization of this mutant indicates that the GmMIPS1 gene is important in the early stages of embryo development.

10 Conclusions

The results found in this review show that EMS mutants generate variation in phenotypic expression, some with importance in agronomy and others in various research areas. One of the advantages found when using EMS is that each mutant line generated increases the genetic variability in the species that are used as study models, allowing plant breeders to have a collection of plants with different characteristics. It is important to note that it is still necessary to continue evaluating the heritability of mutations, in order to obtain new varieties of plants that can be cultivated with uniformity in their genetic response.

Funding Statement: To Consejo Nacional de Ciencia y Tecnología (CONACyT-México) for the support granted through the scholarship for JDJG and the Tecnológico Nacional de México (TecNM) and Instituto de Ciencia y Tecnología e Innovación del Estado de Chiapas for financial support.

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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