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Mutagenic effect of Ethyl methane sulphonate (EMS) on Wheat (*Triticum aestivum* L.)

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Abstract
Wheat (*Triticum aestivum* L.) is the most important crop and is used all over the world in many ways. The ethyl methane sulphonates help in creating the genetic variability in wheat by changing the sequence of nucleotides. Due to which the grain yield increases, and it also provides resistance towards diseases, biological damages and many stress conditions. The chemical mutagen Ethyl methane sulphonate is regarded better than the gamma rays and also better than the physical mutagenesis because of its handling and the physical mutagenesis is also very expensive. So, the chemical mutagen EMS is regarded as best for the mutation process. EMS provides point mutation due to which high-frequency mutation occurs and changes in wheat occurs rapidly. Ethyl methane sulphonate is very useful for the improvement of agronomic traits, screening of elite germplasm and chromosomal aberrations. The ethyl methane sulphonate is used to enhance the genetic variation for wheat improvement. EMS also induces male sterility in wheat plants. It is easily disposable through hydrolysis so this is a better option for breeders and also environmentally friendly that's why it is more preferable. EMS causes a genetic mutation in wheat due to which DNA gets changed so that the changes also occur in offspring and provide high-quality yield. This paper provides complete information about mutations, their effects on wheat, use of Ethyl methane sulphonate in wheat. The study of the Mutagenic effect of EMS in wheat is important for improving the breeding programmes and for better results.

Keywords: Wheat, ethyl methane sulphonate, Germplasm, Mutagenic

Introduction

Wheat (*Triticum aestivum* L.) is regarded as one of the most important cereal crops and a good source of nutrients, food, feed for animals and as a raw material for industries point of view. Wheat has global importance so due to this; it is the most important crop. Due to stress conditions like biotic and abiotic stress causes loss in production and productivity of wheat crop (Bari *et al.*, 1963) [2]. The biotic stress like the incidence of insects, pests and diseases and the abiotic stress like drought stress and loss in soil fertility cause a reduction in the production and productivity of the wheat crop. For the improvement of wheat, the main focus is to develop a high yield potential wheat ideotype, which enhances the root and shoot biomass, protects from stress conditions and increases the carbon sequestration ability in wheat (Lethin *et al.*, 2020) [7]. The change in the genetic makeup of wheat helps in developing the new improved varieties of wheat which can easily tolerate stress conditions and help in the carbon sequestration process for good soil health. The induced mutagenesis helps in making a genetic variation for best breeding results. With the induced genetic variations, in many crops, mutations are also used in many breeding programmes for agronomic traits. For the improvement of pests and diseases, resistance and the improvement of crop yield and quality conventional mutation techniques are used (Kumar & Kumari *et al.*, 2021) [6].

To ensure food security, increasing crop yields is a big challenge. Changing climate (rising temperatures and more unpredictable rainfall), which frequently damage agricultural output, and the need to produce greater food and crops for bioenergy while limiting carbon costs of production, are among the hurdles. As a result, new better yielding cultivars with increased nutrient and water usage efficiency are urgently needed. Mutation breeding has the potential to enhance human health by reducing malnutrition and improving food quality. More than a hundred plant species with many mutant varieties are released officially. The mutant variety of economically important crops like cotton, wheat and barley occupies a lot of cultivable land in the world. The right or accurate selection techniques are most important because it determines the strength of induced variation which are required for the characterization in breeding programmes. The most important way for induced mutations is with the help of chemical mutagens. Among all chemical mutagens, the EMS (Ethyl-methane-sulphonate) is regarded as the best and effective chemical mutagen (Sato & Gaul *et al.*, 1967) [11].

EMS (Ethyl methane sulphonate)

It is an ethylating agent and has a mono function and has several mutagenic effects on mammals and viruses. While on the other hand some of the chemical mutagens are largely manufactured like sodium-azide, and EMS is one of them which have some of the positive effects along with genetic structure. If we talk about the properties of EMS (Ethyl methane sulphonate) at room temperature it is in liquid form and does not have any colour (colourless), the molecular weight of EMS is 124.2 and at 213 to 213.5°c it reaches to boiling point and 1.1452 is the density of EMS, it is more effective as compared to the physical mutagen as it comes under the category of potent alkylating mutagen (Shah *et al.*, 2016) [13]. The group is transferred with the help of the SN1mechanism (SN1- nucleophilic substitution reaction where the rate determining step is unimolecular) which includes substitution, nucleophilic and unimolecular along with SN2 (SN2- one-step reaction in which a nucleophile approaches the substrate and a leaving group, L, leaves at the same time.) which include bimolecular. Due to point mutation, these chemical mutagens can cause both effects such as genotype and phenotype as in crop plants the insertions and removing of changes like genotypic and phenotypic can be beneficial (Mishra *et al.*, 2016) [9]. As the frequency of mutation is enhanced by the chemical mutagens thus, it is preferred by most people and along with this, it is much easier to handle. As the raw material for commercially essential plants is provided by the induced mutation, and also in a short period of time, it can create genetic variability (quantitative and qualitative). As some important traits should be in the field crop such as short period duration, convenient for crop rotation, resistance to biotic and abiotic stress and these traits can be increased by the induced mutations (Kong *et al.*, 2020) [5]. In the DNA phosphate groups, EMS can produce alkylation at oxygen up to some level. With the help of microorganism testing, it has been observed that EMS have the tendency to produce both GC(G-Guaine, C- Cytosine) to AC(A-Adenine, C-Cytosine) and vice versa transition. It has been observed that EMS can break the chromosomes in the higher organism but still the mechanism is not clear. By the action of EMS the DNA bases gradually hydrolyze and leaves out an apurinic and this takes place in the backbone of DNA, but as this site is said to be unstable as it can lead to only breakage of a single strand of DNA but all these are just a hypothesis (Shirasawa *et al.*, 2016) [22]. It is also seen in mice while testing that EMS is the main factor that can break the chromosome according to the data. It has been found that EMS can increase the mutants' proportions such as plaque-type and host range if treated with chemicals in T2 phage as it is the first agent found unambiguously. After this EMS attracts the lots of interest of different scientists and several people also initiate to know about EMS and study the vast range of biological test systems. According to the study conducted by the Environmental Mutagen Information centre (EMIC) it is found that there are more than 3400 chemical references in which the EMS role is seen to be only as a positive control and also EMS helps is creating new mutants (Bahar & Akkaya *et al.*, 2009) [1]. The structural formula of EMS is shown in (figure 1).

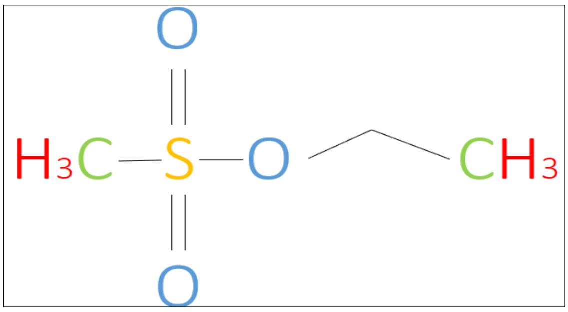

Fig 1: Structural formula of EMS ($C_3H_8SO_8$)

Efficiency and effect of EMS on wheat (*Triticum Aestivum*)

If we talk about the effect of mutagen in the plant it means that how much-desired effect is produced by the mutagen and the effect can be both, positive or negative. But, EMS (Ethyl methanesulfonate) is a very effective chemical mutagen to enhance the point mutation and it has also been seen in chickpea. In combined treatments it is observed that as compared to gamma rays, EMS is very effective and especially in various programmes of breeding it is preferred as the application is very convenient and along with this the penetration is much better, frequency of mutation is also very high, problem related to disposal is also less (Lethin *et al.*, 2020) [7]. The toxicity of EMS may vary according to the species and presence of any other mutagens and also post-treatment with antioxidants. To increase the rate of genetic variability it is very important to induce mutations as in both micro and higher organisms. EMS induce a very high rate of mutations as sometimes the frequency can be much high than it can be obtained. For the mulberry plant, EMS is found to be much potent than gamma rays to build up the new varieties and increase the yield. In different plants, the role of EMS in different like in tomato EMS acts on the leaves and reduce the size of the fruit and build the plant resistant to many diseases and in capsicum, the antibiotic-resistant variant was isolated (Shimelis *et al.*, 2019) [14]. In medicinal and economic plants it has been observed by the workers that the genetic variability is enhanced by the chemical mutagens. EMS has more effect on the chlorophyll content of plants and also it projects the abnormalities in plants such as univalent, multivalent, stickiness.

EMS in wheat (*Triticum Aestivum*)

As per the increasing population day by day it is the need of today to improve the quantity of wheat because quantity can be improved easily it is not an issue and people are already doing that but the main thing is to improve the quality which can be done by genetic mutations. To create a proper genetic variation conventional breeding techniques is needed. To achieve the conventional breeding method it is important to practice rapid methods i.e. mutation breeding, during the replication of DNA mutations can occur and at the time of reproduction, the material which is present in DNA can be passed to offspring (Sega *et al.*, 1984) [12]. For breeding purposes, natural mutagens are not that much effective as they are minor but they can be useful by introducing chemical mutagens, but due to very expensive equipment, the physical mutagens are only used by developed countries. On the other hand, for the chemical mutagen, expensive equipment is not required. Due to the high mutation rate of EMS especially it is good at point mutation it gained more interest for the mutation breeding of wheat (Bonchev *et al.,* 2010) [3]. There are different types of chemical mutagens and EMS is one of them which can induce mutation and the chemical properties on EMS decide the desirable mutation along with this environmental factor is also responsible. To create a new variety there is a requirement of high-frequency mutation so that the mutation will be much rapid and easy without creating any biological damage, and EMS is preferred for the mutation as it has high frequency and is very effective. By the substitution of nucleotide, EMS produces random mutation in the genetic material. The main benefit is that it can easily be disposable by hydrolysis and does not have any negative effect on the environment (Olaolorum *et al.,* 2021). The grain quality of wheat is increased by the EMS mutagenesis and also it makes the plant disease and herbicide resistance also the male sterility is induced by EMS. The morphological variation can be seen when EMS is introduced, some of the mutant varieties in wheatsuch as Deada, Emai 23, Darkhan 106 and Baichun 5 are the improved version of wheatwhich have a very high grain yield and better traits as compared to verities which are produced conventionally. Mutagenesis also improves the root traits because more attention is given to the shoot part of the plant but generally people forget that root is the most important part of the plant and thus, it needs more attention and by mutagenesis, the root is improved (Lombardo *et al.,* 2017) [8]. In wheat (*Triticum Aestivum*), EMS change the nucleotide sequence thus automatically changing the DNA information. EMS can easily induce the same types of mutation in both diploids and polyploids. Ethyl-methane-sulphonate is the most powerful and effective chemical mutagen mainly used for seed materials along with this its application is also easy. The EMS causes point mutations in plants so that the deletion and loss of chromosome segments occur to a very less extent. Without inducing large deletions, EMS can alter the loci of specific interest. Due to this, a plant breeder can easily obtain the alleles which are useful (Hailegiorgis *et al.,* 2018) [4]. This is a great advantage for plant breeders because in wild germplasm many exotic or deadly alleles are also present so with the help of EMS plant breeders can easily use the useful alleles. While doing work with EMS, the most important point to be remembered for induced mutation is the use of the right concentration of EMS, the temperature of the solution and the time duration of the treatment. Many researchers are working on a large scale on induced mutations, the large-scale generation of variants are used to find the combination of all the parameters with the liable doses for getting the best response (Bari, G*et al.,* 1963) [2]. Among all the mutagens, the chemical mutagens and the physical mutagens both are tested in many different crop species of cotton, barley, corn, vegetables, tobacco, rice, fruits and oilseed crops. During the mutagenesis process, the treatment of Ethyl-methane-sulphonate or the gamma rays makes the plant vulnerable to the negative effects of M1 characters like production of seeds, length of seedlings, seed germination and root length etc. The optimization is very important for making a large number of mutants without any genetic change and having good seed germination for segregation processes (Mishra *et al.,* 2016) [9]. With the use of mutagenesis in genetic variation for breeding, the induced mutation is also used for hindering gene functions that cause phenotypic changes in basic research. For confirming the function of a gene, diseases resistance mechanism, developmental and biochemical elucidation is important. Mainly the mutations with induced mutants are through Ethyl methane sulphonate are the type of point mutations having a good range of alleles in a very small population (Kong *et al.,* 2020) [5]. In this, the genetic changes occur at the nucleotide level of the candidate gene this will cause a loss in the function and can be auto-generated by not losing the genes. The application of selection methods accurately is well known for the characterization of tilling and phenotyping experiments. The genes involved in the disease resistance are the candidate genes and can easily be confined by a particular trait. During phenotypic change, most of the mutations come under Pleitropic mutations because some plant organs like leaves are the most prone parts towards alterations (Kumar & Kumari., 2021) [6]. Every mutant belongs to some category and has some definite function like delay in germination, stunted growth, narrow leaves, wider leaves, rough leaves, smooth leaves, the small size of plants, lethal embryo, early flowering, late flowering, sterility and different leaf colour like purple, green, dark green, yellow and white etc. Many researches found that the plants that belong to M1 and M2 generations have less rate of survival, less fertility and delayed seed and fruit emergence. It is also proved from many studies that the treatment of Ethyl methane sulphonate was more effective than the treatment of gamma rays (Olaolorum *et al.,* 2017). In the research work on *Vicia faba,* it was found that the treatment of Ethyl methane sulphonate affects the seedling parameters and germination processes by mutagenesis process. The frequency of seedlings gets reduced, germination slows down and the length of plumule and radicle also reduces. Along with this with the effective mutagenesis, physiological injuries are found in the radicle part of the plant (Bonchev *et al.,* 2010) [3]. The mutagenic treatment also depends upon the dose and causes stress like reduction in pollen fertility, seed germination, late maturity of the fruit and flower, less survival rate. For producing wheat varieties with special characters like stress resistance, high nutrient availability etc. the molecular understanding of these processes is important. The molecular mechanism used for the tolerance of stress is now getting in the face. There are various molecular genetic approaches are used but these two are very effective and important as well. These genetic approaches are Transgenic breeding and Marker-assisted breeding. Marker-assisted breeding to have the various advantage that it can target a particular allele with proper precision (Bahar & Akkaya *et al*., 2009) [1].

Table 1: Shows the wheat Triticum aestivum and mutagen and concentration

S. No.	Wheat (*Triticum Aestivum*)	Mutagen	Mutagen Concentration	Traits	Effect	References
1	*Triticum aestivum* L. 'Jing411'(Cultivar)	EMS	0.5, 1.0, or 1.5% EMS in 0.1 M sodium phosphate buffer (pH 7.0)	high yield potential, cold tolerance, high tilling ability	A new genetic resource with phenotypic variation and non-lethal alleles was developed using a hexaploid winter wheat cultivar as the wild type. This resource could be used for wheat forward and reverse genetic research to improve wheat agronomic traits.	Guo *et al.*, 2017
2	*Triticum aestivum* L. "Gerek-79"(Cultivar)	EMS	Three different percentages of EMS doses (0.10%, 0.20%, 0.30%) and a control dose (0% EMS) were applied.	coleoptyl existence, embryonic root length and coleoptyl length, first leaf existence, first leaf length, seedling growth rate, fresh and dry weights of shoots and roots, root to shoot ratio	All observed traits related to shoots and roots were decreased by increased EMS doses.	Bahar *et al.*, 2019 [1]
3	*Triticum aestivum L.* 'NN-Gandum-1'(Cultivar)	EMS	optimized concentration of 0.8% (v/v) of EMS	biochemical and physiological assays	The selected mutants under drought stress increased the accumulation of proline content, total soluble sugars, total free amino acids, while decreased total chlorophyll content, carotenoids and total soluble protein. Finally, the procedure of narrowing down the number of developed mutants from a large mutation population (>4000) is found useful for exploring the complex trait like drought without compromising yield potential.	Zahra *et al.*, 2021 [16]
4	*Triticum durum* 'Cappelli'(Cultivar)	EMS	1. EMS 1.5% with Cu 10-3M 2. EMS 1.5% with Cu10-3mM 3. EMS 1.5%	cytological studies (number and percentage Of aberrant anaphases and the number of chromosomal breaks per anaphase) of the primary root tips, and study on root and shoot length of the seedlings	There is a considerable increase in the number of aberrant anaphases and in the number of chromosomal breaks per anaphase with the addition of copper to ethyl methanesulphonate, The root and shoot lengths are maximum in case of treatments with both doses of copper alone, and with the higher close there is a relative increase in the seedling length.	BARI *et al.*, 1963 [2]
5	*Triticum aestivum L.* 'Ningchun 4', 'Aikang 58', 'Lunxuan 987', 'Zhoumai 16', and 'Jing RS801' (Cultivars)	EMS	0.5% (v/v) EMS	IMI-resistant wheat mutants	various degrees of IMI resistance to wheat plants and provides a foundation for understanding IMI resistance and breeding wheat varieties with herbicide resistance,	Chen *et al.*, 2021 [17]
6	*Triticum aestivum L.* 'Sonora 64' (cultivar)	GAMMA RAYS AND EMS	Gamma Rays- The doses, *viz.*, 20 and 30 kr were given. EMS- Seeds presoaked in distilled water for 14 hours were given 8 (EMS 1/100 and EMS 1/200) as well as 16 (EMS 1/200 and EMS 1/400) hours treatment.	Comparison of the spectrum and frequency of mutations induced bygamma rays and ethyl methane sulphonate.	It is found that EMS is capable of inducing a wide range of mutations with a higher frequency. It is suggested that in polyploid crops, chemical mutagenslike EMS may prove more advantageous than physical mutagens for the induction of qualitative changes.	Varughese *et al.*, 1968 [18]
7	*Triticum aestivum* L. 'OW 32-3 and OW 13-1' (cultivar)	Ethylmethane sulphonate (EMS) and sodium azide (SA)	Ethyl methane sulphonate (EMS) at 0.5% and 0.25% and sodium azide (SA) at 0.02% and 0.01%.	Variability in pollen grain size, pollen sterility.	Significant increase in pollen sterility with increase in dose of the mutagens as compared to the controls. SA treatments increased the variability of pollen grain size in both the varieties, whereas the	Subudhi *et al.*, 1991 [19]

					EMS treatments increased variability in OW 13-1 and decreased in OW 32-3.	
8	*Triticum aestivum* L. *Triticum monococeum* var. Japanese Early (2n=14), *T. dicoccum* var. Khapli (2n=28), *T. aestivum* varieties C. 591 and H. 389 (2n=42), *Hordeum vulgare* var. N,P. 13 (2n = 14) and colchicine-induced autotetraploid *H. vulgare* var. N.P. 13 (2n=28)	EMS	Seeds were soaked in EMS solutions of strength 180, 220, 280 and 400 ppm. using about ten times more solution than the corresponding volume of the seeds.	Mitosis in root-tip, Survival, morphological changes and seed fertility, and Chromosome aberrations at meiosis.	EMS hardly ever induces chromosome breakage. Chlorophyll mutations were abundant in the M2 generation. Viable mutations also occurred at a high frequency in 2x barley but were rare or absent in the wheat species.	Swaminathan *et al.*, 1962 [20]
9	*Triticum dicoccum* 'Khapli' (Cultivar)	EMS and HA (Hydroxylamine)	EMS- 0·4 per cent. and 0·6 per cent. HA- 0·25 percent and 0·5 percent	Compare the frequency and spectrum of mutations induced by individual and combined treatments of EMS and HA.	HA proved to be a weak and EMS proved to be a potent mutagen both in normal plants of Khapli as well as in an induced chlorina mutant.	Chopra *et al.*, 1966 [21]
10	*Triticum durum* 'Cappelli' (cultivar)	EMS	EMS- 1.5% with Cu (10-3M) EMS- 1.5% with Cu (10-3mM) EMS 1.5% Cu (10-3M) Cu (10-3mM)	chromosomal aberrations and cytological study	Ethyl methane sulphonate becomes more effective in inducing chromosomal aberrations when growth is stimulated.	Bari *et al.*, 1963 [2]

Conclusion

Triticum aestivum commonly known as wheat is one of the oldest crops and is used all over the world. Wheat is regarded as the most important cereal crop for grains production and provides a good source of nutritious food for human beings. Wheat is grown on a large scale than other crops. In India, wheat is grown on a very large scale than any other crop. Now a days the human population is increasing day by day and due to industrialization, the soil salinity becomes more prevalent so for meeting the demands for good grain quality and higher yield, salt-tolerant varieties are made, due to this, there is increased pressure on wheat breeders. Mutation breeding is the most effective and highly used technique for the improvement of wheat. Chemical mutagenesis is also very effective, in this the most common mutagen is Ethyl methane sulphonate (EMS). This EMS mutagen is mainly used to induce the mutagens with some agronomic traits in different crops. The EMS provides point mutations, due to which the screening of mutants gets easy. The chemical mutagenesis with EMS provides an increase in crop growth, yield and quality of the crop. The root length, shoot length, flowering, fruiting, resistance power towards many stress conditions also increases. This review paper provides the proper information about the mutagenesis effect in wheat with the help of Ethyl methane sulphonate, its importance, working and its application.

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