# Mutagenic Action of Ethyl Methanesulphonate (EMS): A Review

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**Abstract**
EMS is considered as both dangerous mutagenic and carcinogenic agent, however agriculturists and biotechnologists use it for crop improvement vis a vis agricultural production. It is known as easily available mutagen having less impact on biological systems to produce new and novel mutants with desirable characters in a large variety of genetic test systems. EMS is used frequently and abundantly in plant systems as it causes a high frequency of nucleotide and substitution variations. In this review, our focus will be on the role of chemical mutagens and the biological mechanisms for inducing the variations in crop plants and the future perspective in the advancement of agricultural biotechnology.
**Keywords:** EMS, Genetic variation, mutation.

## Introduction

Ethyl methanesulfonate (EMS) is considerd as a monofunctional ethylating agent which has shown its mutagenic effect in a large variety of genetic test systems ranging from viruses to mammals (Meuth *et al.,* 1982). However, chemical mutagens have been manufactured at a large scale , such as sodium azide, ethyl methanesulphonate (EMS) and *N*-ethyl-*N*-nitrosourea (ENU), which show different side effects and positive effects as well as on the genetic structure of mutated populations ( Mohd-Yusoff *et al.,* 2015). EMS is a colorless liquid at room temperature and its molecular weight is 124.2. The boiling point of EMS is 213-213.5°C (761 mmHg) and its density is 1.1452 at 22°C relative to water at 4°C (IARC, 1974). EMS being a potent alkylating mutagen and sometimes more effective than physical mutagens (Bhat *et al.,* 2005). EMS owes its biological reactivity to its ethyl group. The transfer of the group occur via SN1 (substitution, nucleophilic, unimolecular) or an SN2 (substitution, nucleophilic, bimolecular) mechanism (Osterman-Golkar *et al.,* 1970).The main causes are point mutations which are single-base substitutions and may arise due to transitions i.e purine to purine or pyrimidine to pyrimidine and transversions (pyrimidine to a purine) (Ennis, 2001). EMS alkylates guanine bases and due to this mispairing- alkylated G pairs with T instead of C, resulting in primarily G/C to A/T transitions but apart from this it also can cause small deletions and rearrangements depending on the position of the mutation (Rafi *et al.,* 2016). These chemicals mutagens can cause phenotypic effect as well as genotypic effect in the genomic strands due to point mutations, insertions and or deletions leading to the phenotypic and genotypic changes which could be beneficial for crop plants (Greene *et al.,* 2003; Flibotte *et al.,* 2010).Chemical mutagens are in high demand as they increase mutation frequency and are easier to handle (Sikora *et al.,* 2011; Serrat *et al.,* 2014). Induced mutation provides raw

materials for the genetic improvement of commercially essential plants (Adamu and Aliyu, 2007) and also used to create genetic variability in a short period of time in quantitative as well as qualitative traits (Devi and Mullainathan, 2012; Tshilenge- Lukanda *et al.*, 2013; Aruldoss *et al.*, 2015). Agronomically important traits such as shorter growing period, suitable for rotation, increased tolerance or resistance to abiotic and biotic stresses are developed with the help of induced mutations (Monica and Seetharaman, 2016).

**How mutation takes place?**

The DNA is believed to be the most important target for the induction of mutations by chemical agents and many studies have focused on the interactions between DNA and chemical mutagens.

EMS, an alkylating agent, is commonly used as a chemical mutagen for DNA lesions and induces base changes or nucleotide substitution, which consequently alter codon sequences, leading to either nonsynonymous or synonymous effects also and induces a biased spectrum of G/C-to-A/T transitions and these transitions occur due to the alkylation at the $O^6$ or $N^7$ position of guanine, which leads to the replacement of cytosine with thymine base pairing (Sikora *et al.*, 2011). Originally, it was found that, the *N-7* of guanine was the first site ethylated by EMS (Brookes and Lawley, 1961). This is also the predominant site of attack by EMS in DNA. Because of the high occurrence of *7*-alkylguanine residues formed in the reaction of alkylating agents, such as EMS, with DNA, mispairing of this modified purine was once believed to be an important cause of mutations (Auerbach, 1976).

Other workers had recommended that *7*-alkylguanine may not be of great significance in the mechanism of mutagenesis and carcinogenesis of alkylating agents (Montesano and Bartsch, 1976). For example, guanine alkylated at the *N-7* position is not likely to be involved in mispairing (Koch and Miller, 1965). Also, effective mutagenic agents, like EMS and *N*-methyl-*N*-nitrosourea (MNU), and weaker mutagens, such as methyl methanesulfonate (MMS) and dimethyl sulfate (DMS) are both effective in alkylating the *N-7* position of guanine (Singer, 1982). Loveless (1969) was the first to recommend that mutagenicity may be correlated with the formation of $O^6$-alkylguanine other than of *N-7*-alkylguanine. He observed indication for $O^6$-alkylation by EMS and MNU but MMS and DMS showed very less mutation regarding $O^6$-alkylation. Other workers had also found that chemicals reacting through an SN~ mechanism produce higher amounts of $O^6$-alkylguanine relative to *N-7*-alkyiguanine than do chemicals reacting by an SN2-type mechanism (Lawley and Thatcher, 1970). Thus, EMS, which can react by an SN1 mechanism as well as an SN2 mechanism, is expected to produce relatively more $O^6$-ethylation than that found with MMS which reacts via an SN2-type mechanism (Lawley and Thatcher, 1970). Mechanism of EMS begins with the action of mutagen that breaks the nuclear DNA during DNA repair mechanism (Gupta *et al.*, 2016). The high frequency of G/C-to-A/T changes has been observed upon EMS exposure in different organisms including *Arabidopsis thaliana* (Greene *et al.*, 2003; Till *et al.*, 2011), *Oryza sativa* (Henry *et al.*, 2014: **Figure 1**), *Japonicas* (Perry *et al.*, 2009), *Caenorhabditis elegans* (Flibotte *et al.*, 2010; Thompson *et al.*,2013), *Solanum lycopersicum* (Minoia *et al.*, 2010) and *Saccharomyces cerevisiae* (Shiwa *et al.*, 2012) at different rates.

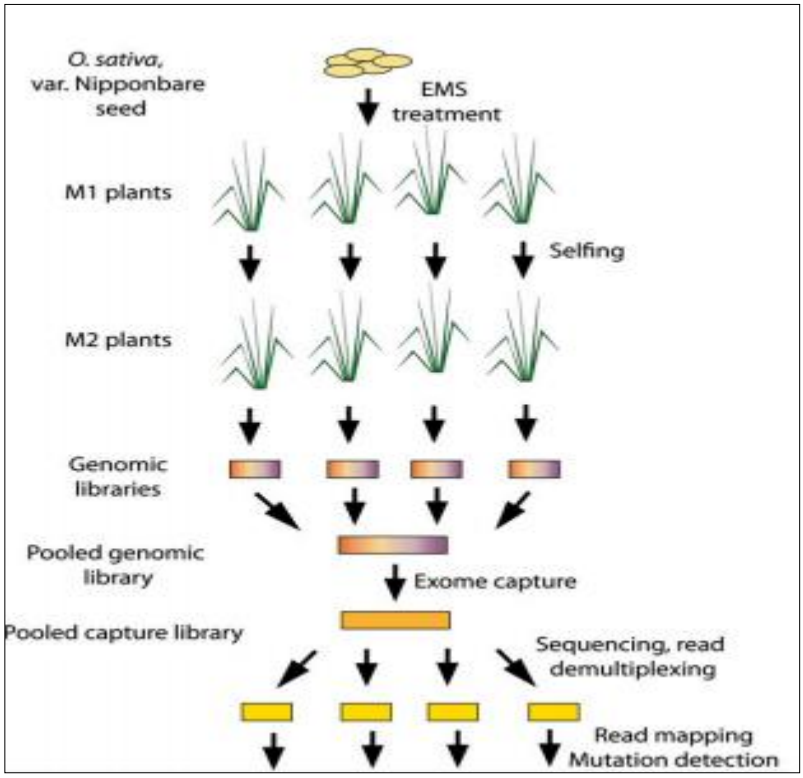


**Figure 1: Production and Analysis of the EMS mutagenized Rice Samples (Henry *et al*., 2014)**

**Efficiency and effect of Ethyl methanesulphonate (EMS) on plants:**

Effectiveness means the degree to which mutagen is successful in producing a desired effect which may be positive or negative. However, ethyl methanesulfonate (EMS) is considered to be an effective and extensively used chemical mutagen to induce point mutations (Till *et al.,* 2004). Khan *et al.* (2005) had reported the order of mutagenic effectiveness in chick pea as Hz> SA> EMS. Durdana, 2016 observed that lower concentration of EMS are more effective than higher concentrations in *Hyoscyamus niger*. EMS was found to be more effective than gamma rays and in combined treatment (Wani, 2009). In breeding programmes EMS has been extensively used because of their simple application, good penetration, reproducibility, high mutation frequency and less disposal problems (Chahal and Gossal, 2002). Depending upon the species and other mutagens or post-treatments with antioxidants, toxicity of EMS may vary (Henikoff and Comai, 2003). The dose assessment of chemical mutagens is determined by varying the concentration and duration of treatment, solvent used or pH of the solution (Jain, 2010). Induced mutations are necessary to enhance the rate of genetic variability. (Gupta *et al.,*2016). EMS induce a high rate of mutations in both micro and higher organisms and sometimes the mutation frequencies exceed those obtained by radiation (Goud, 1967). Of all the mutagens accessible nowadays, gamma rays and EMS have been found more potent for mulberry (Deshpande *et al.,* 2010). Khatri *et al.* (2005) reported that EMS could be fruitfully useful to build up new varieties with high yield and other improved organic traits. High frequency of EMS for producing phenotypic variation like potato shaped leaves, reduced fruit size, and maximum disease resistance were observed in tomato (Yudhvir, 1995). High frequencies of plastid-encoded antibiotic-resistant variants were isolated in *Capsicum annuum* (Rao *et al.,* 1997). Large number of workers have revealed that the role of chemical mutagens in increasing genetic variability in medicinal and economical important plants (Coe and

Neuffer, 1977; Mashenkov, 1986; Ricardo and Ando (1998). Alkylating agents such as methyl methanesulfonate (MMS), Ethyl methanesulphonate (EMS); *N*-methyl-*N*-nitrosourea (NMU), Ethylnitroso-urea (ENH) and Methylnitroso-urea (MNH) are commonly used chemical mutagens for induction of variability in plants for improvement of important characteristics. EMS was found to be successful in protruding abnormalities like sticikiness, univalent, multivalents, laggards and bridges as well (khan and Tyagi, 2009) and has shown deep effect on chlorophyll content. Ethyl methanesulphonate (EMS) is used widely to induce a higher frequency of mutations in crop plants (Kozgar *et al.*, 2011; Jagajanantham *et al.*, 2013; Kashind and More, 2016). It typically causes high frequency of gene mutations and low frequency of chromosome aberrations, but loss of a chromosome segment or deletion is also reported in many plants (Van Harten, 1998; Khatri *et al.*, 2005).

**Conclusion**

Using EMS as mutating agent on the plant genome as it has a potential to generate many new mutants with desirable characters, in a wide variety of genetic test systems. EMS has been widely used to introduce a large number of functional variations in many crop plants and most frequently used chemical mutagen, as it can cause a high frequency of nucleotide substitution variation, as detected in different genomes.

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