Journal of Oilseed Brassica, 7 (1) : 77-82, January 2016

Determination of LD_{50} of ethyl methanesulfonate (EMS) for induction of mutations in rapeseed-mustard

Prashant Yadav, HS Meena*, PD Meena, Arun Kumar, Riteka Gupta, S Jambhulkar[1], Reema Rani and Dhiraj Singh

ICAR-Directorate of Rapeseed-Mustard Research, Bharatpur-321303, Rajasthan, India
[1]Bhabha Atomic Research Centre, Trombay, Mumbai-400 085, Maharashtra, India
**Corresponding author: singh_hari2006@yahoo.co.in*
(Received: 22 Nov 2015; Revised: 22 Dec 2015; Accepted: 27 Dec 2015)

Abstract

Mutation breeding is an effective way to enrich genetic variability in crop plants. There are two basic means, physical and chemical mutagens for inducing mutations. Among chemical mutagens, the alkylating agent, ethyl methanesulfonate (EMS) is the most commonly used mutagen in plants as it causes a high frequency of nucleotide substitutions i.e. point mutations. Hence, an optimum dose is highly desired to produce the high frequency of mutations with minimum killing of treated individuals. Therefore, the present investigation was undertaken to determine the LD_{50} of EMS and effect of different dosages of EMS on seed germination of two Indian mustard varieties (viz. RH-749 and NRCHB-101) and one of its important wild relative *Sinapis alba*. Results revealed the significant effects of EMS dosages and treatment periods on seed germination. The EMS doses (LD_{50}) at 0.42%, 0.73% and 0.3% for duration of 12 h were found to be optimum for Indian mustard varieties (RH-749, NRCDR-101) and *S. alba* respectively. The LD_{50} of EMS for *Brassica juncea* was higher than the *S. alba* and it also varied for two varieties of *B. juncea*. This information would be highly useful for initiating mutation breeding programme in rapeseed-mustard crops.

Key words: *Brassica, EMS, LD_{50}, mutagenesis, Sinapis alba*

Introduction

Rapeseed-mustard crops, particularly the Indian mustard has a highly significant role in Indian agriculture. It is second important oilseed crop at national level contributing nearly 1/3rd of the edible oil pool of the country (Pratap *et al.*, 2014). Considering the population growth rate, increased per capita edible oil consumption and mounting imports of vegetable oils, rapeseed-mustard crops signifies a positive trend and offers enormous scope for yield enhancement on long term basis. In spite of achieving impressive productivity gains through development of many improved cultivars; still there is compelling need to further increase and stabilize the productivity of this crop (Meena *et al.*, 2015). To enhance the production of any crop, a good variability should be present in the primary gene pool (Kumar *et al.*, 2013a; Kumar *et al.*, 2015). Genetic variation plays a critical role in developing well-adapted improved cultivars. Since, there is limited genetic variability in primary gene pool of *Brassica juncea*; the various tools to generate new genetic variability shall be employed. However, cross breeding has restricted usages due to limited genetic variability in nature (Sestili *et al.*, 2010). Mutation breeding might be the effective alternate to augment genetic variation, particularly for traits having low level of genetic variation (Szarejko and Forster, 2007). Numerous reports on successful mutation breeding are available in various oilseed crops (Bacelis, 2001; Spasibionek, 2006; Ferrie *et al.*, 2008; Parry *et al.*, 2009). Induced mutations have been used mainly to generate variation that could rarely be found in germplasm collections. Mutagenesis have been employed to improve a large number of desirable characters like earliness, dwarfness, biotic and abiotic stress resistance, seed yield and oil quality (Schnurbush *et al.*, 2000; Parry *et al.*, 2009). Many physical and

chemical mutagens are available to induce mutations in crop plants. The nature of alteration in genetic makeup of crop plant depends on the specific mode of action of a particular mutagen (Feldmann *et al.,* 1994; Meinke *et al.,* 1998). Several rearrangements in DNA fragments may occur based on dose level and treatment time of various mutagens that may result in production of a range of mutants. The information on right dose of a particular mutagen for specific crop, species or genotype is highly important to induce mutants with desirable frequency. Ethyl Methane Sulphonate (EMS) is a chemical mutagen, produces random mutations in plant genome and is reported to be the most effective and powerful mutagen (Minocha & Arnason., 1962; Hajra 1979) that typically produces only point mutations (Okagaki *et al.,* 1991). In Brassica crops, Fowler and Stefansson (1975) obtained many morphological variations using EMS in *B. napus*. Similarly, Khalatkar and Indurkar (1991) reported various mutations for "00" genotypes in *B. juncea* and early flowering mutant in *B. napus* have been reported by Landge and Khalatkar (1995). Usually, mutagen treatments reduce seed germination, growth rate, vigour and fertility. There is substantial killing of plants during different stages of development, thus considerably reduces the survival of resulting plants. The dose required for high mutagenic efficiency depends on properties of the mutagenic agent and material treated. Hence, an overdose might kill too many treated individuals and an underdose will produce fewer mutations. T**he** optimum dose will produce the high frequency of mutations and cause minimum killing. Many workers felt that a dose near to LD_{50} should be the optimum which varies with crop species and mutagen used (Singh, 2000). Therefore, the information on suitable dose of a particular mutagen for a specific crop, various species and different cultivars of a same species is highly useful. In Rapeseed-mustard crops, there are limited information on optimum dose of chemical mutagens and the reports on species wise and cultivar differences are not available. Therefore, keeping the above points in view the present investigation was conducted to determine the LD_{50} of EMS for Brassica species and to determine the effect of ploidy levels (diploid and tetraploid) of the species and different varieties of the same species on optimum dose of chemical mutagen (EMS).

Materials and Methods

The present study was conducted at ICAR-Directorate of Rapeseed-Mustard Research, Bharatpur (India) during 2014-15. The experimental material consisted of two different species of *Brassica* having two different ploidy levels *viz., Sinapis alba* (Diploid, 2n=2x=24, SS) and *B. juncea* (Tetraploid, 2n=4x=36, AABB) and two varieties of the same species *B. juncea* (Indian Mustard) viz. RH-749 & NRCDR-101. Ethyl methanesulfonate (EMS) was used as chemical mutagen for induction of mutation. The study was conducted under controlled conditions in laboratory with three replications. Hundred seeds of each genotype for each replication were first presoaked in distilled water for 2 h and then treated overnight with 8 different concentrations of EMS (0.1%, 0.25%, 0.5%, 0.75%, 1.0%, 1.25%, 1.5% and 2%) for 12 h at 24 °C with constant shaking at 100 rpm. The overnight treated seeds were washed 3-4 times under running tap water to remove residual EMS from the seeds. The seeds were placed on petri dishes on distilled water soaked whatman paper disks. The seeds for control were treated with distilled water only. Petri dishes were placed on tissue culture racks for seed germination. The room temperature was maintained at 25 ± 2 °C. Distilled water was constantly applied twice a day with 8 h differences to maintain moisture in the paper. All sprouted seeds were considered as germinated either the resulting seedlings were normal or abnormal. Observations were recorded on 3rd and 6th days after treatment (DAT), the germinated seeds were counted from each petri plate. Percent seed germination was calculard using average of the three replications and data was analyzed for determining LD_{50}.

Results and Discussion

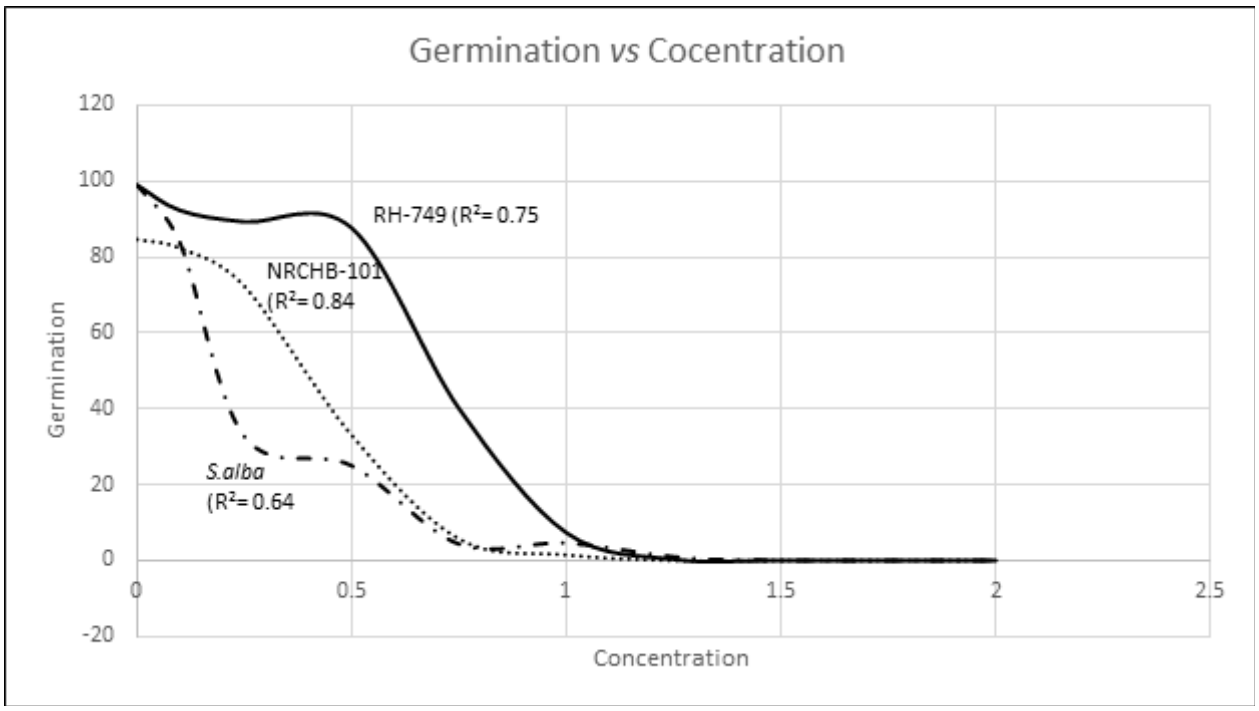
Mean germination percentage of *S. alba* and *B. Juncea* (Var. RH-749 & NRCDR-101) genotypes at 8 different doses of EMS is presented in Table 1. The germination was recorded on 3rd and 6th DAT and the LD_{50} was calculated on the basis of percent germination on 3rd day. The seedlings having normal root and shoots were considered for

Table 1: Effect of different doses of EMS on germination of Indian mustard varieties (RH-749 & NRCHB-101) and *S. alba*

Dose of EMS	C**	0.10%		0.25%		0.50%		0.75%		1%		1.25%		1.50%		2%	
DAT*	3	3	6	3	6	3	6	3	6	3	6	3	6	3	6	3	6
RH-749	91	82	94	72	85	33	62	6	18	2	10	0	0	0	0	0	0
NRCHB-101	98	92	96	89	95	88	94	40	76	7	58	0	6	0	0	0	0
S. alba	99	84	93	33	68	25	57	4	37	5	28	1	9	0	0	0	0

*Days after treatment; **Control

Fig 1: Decrease in germination percentage with increasing concentrations of EMS in *B. juncea* (RH-749 & NRCHB-101) and *S.alba*



calculating the LD_{50} as most of the seedlings from delayed germinated seeds were abnormal. The results indicated that the average germination was decreased with increasing in the concentration of mutagen (EMS). The percent germination was reduced from 91, 98 and 99 to 2, 7 and 5 percent with 1% of EMS for genotypes RH-749, NRCHB-101 and *S. alba*, respectively. At the same time, almost all the genotypes showed zero (0.00%) germination at the concentration of 1.25% and higher doses of EMS. This revealed that the doses of EMS higher than 1% are highly lethal irrespective of genotype and species. The germination percentage on 6th day after treatment (DAT) was found higher than the germination percentage on 3rd DAT indicating the delay in seed germination due to effect of mutagen treatment (Fig 3). Most of the seedlings emerged from delayed germinated seeds were abnormal which could not developed into normal seedlings. This showed the effect of mutagenic treatment on seed germination and its lethality. Delayed germination was observed with most of the EMS dosage (Fig 3).

Fig 2: Differences in LD50 of EMS in *B. juncea* (RH-749& NRCHB-101) and *S. alba*

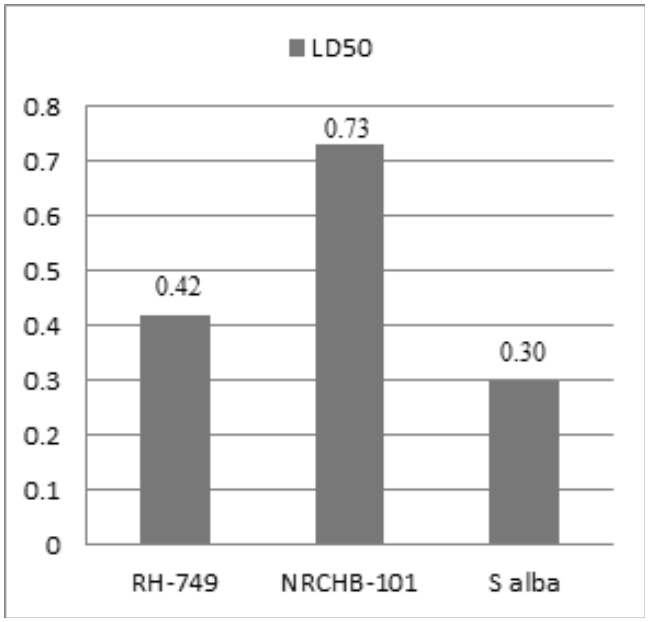
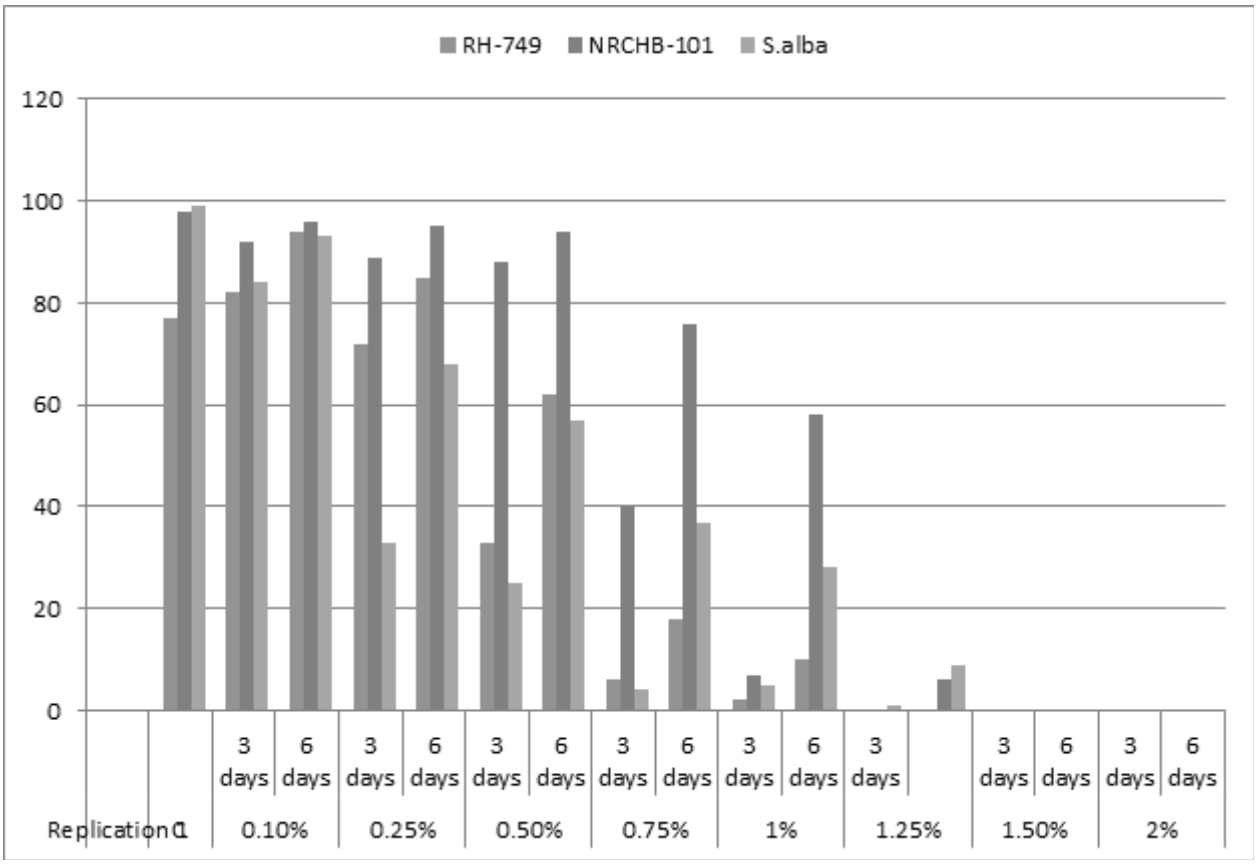


Fig 3: Effect of EMS on delayed germination of Indian mustard varieties (RH-749 & NRCHB-101) and *S. alba*



Induced mutagenesis is a tool to create new variability in any crop with a higher frequency than the spontaneous mutations (Chopra, 2005). For artificially induced mutations either with physical or chemical mutagens, LD_{50} has been considered as the best dose for high frequency of mutations (Anbarasan *et al.*, 2013). In the present investigation the LD50 was calculated on the basis of seed germination at different doses of EMS. The LD_{50} of two *B. juncea* varieties RH-749 and NRCHB-101 was found at 0.42% and 0.73% of EMS dosages respectively (fig. 1). Similarly, Khatri *et al.*, (2005) reported the optimum concentration of EMS in *B. juncea* cv. S-9 ranging from 0.7% to 1% with a treatment period of 3-4 hours. The variation in the LD_{50} of EMS for the two varieties of the same species was found different, suggesting that it may vary from one genotype to another (fig. 2). It might be due to the differences in their genetic constitution and their parentage. Similarly, the LD_{50} for the *S. alba* was found nearly 0.30% which was lower than the LD_{50} of both the genotypes of *B. juncea* (fig. 2). It shows that the LD_{50} for *B. juncea* is higher than the *S. alba*. The provable reason for the higher LD_{50} for *B. juncea* genotypes compared to *S. alba* might be due to the differences in their ploidy level as the tetraploid genome of *B. juncea* may have higher buffering capacity towards mutagenic effects as compared to diploid genome of *S. alba*. Emrani *et al*. (2011) reported 0.8% EMS concentration optimum (LD_{50}) for *B. napus* which is again higher in comparison to our results with *B. juncea* genotypes. However, they treated the seeds for 6 hours in EMS in contrast to the present study (12 hours) and may be the longer treatment period requires lesser dose of EMS, besides the genotypic difference this may also be a reason. Furthermore, these two species are amphidiploid (tetraploid) in nature but the number of chromosomes in *B. napus* (2n=38, AACC) are higher than the *B. juncea* (2n=36, AABB) and have one different genome. Kumar and coworkers (2013b) reported 0.6% dose of EMS as LD_{50} with 8 hours of treatment period in *Helianthus annuus*. Similarly, Talebi and coworkers (2012) reported the LD_{50} of EMS in Malaysian rice as 0.5% with 12 hour of treatment period. The information generated in the present investigation may be highly useful for initiating mutation breeding programmes in rapeseed- mustard crops as the LD_{50} of a particular mutagen for a particular genotype may not be the same for all the genotypes of the same species or for different species of the same genus.

Acknowledgements

Authors are grateful to the Board of research in Nuclear Sciences (BRNS), Government of India for providing financial assistance in the form of BRNS project entitled "Induced mutagenesis for isolation of Alternaria blight resistant mutant in *Brassica juncea*" bearing sanction No. 35/14/44/2014-BRNS and Director, ICAR-DRMR for providing laboratory facilities.

References

Anbarasan K, Sivalingam D, Rajendran R, Anbazhagan M and Chidambaram AIA. 2013. Studies on the mutagenic effect of EMS on seed germination and seedling characters of Sesame (*Sesamum indicum* L.) Var.T MV3. *Int J Res Biol Sci* **3:** 68-70.

Bacelis K. 2001. Experimental mutagenesis in fiber flax breeding. *Biologia* **1:** 40-43.

Chopra V L. 2005. Mutagenesis: Investigating the Process and Processing the Outcome for Crop Improvement. *Current Science* **89:** 353-359.

Emrani SN, Arzani A and Saeidi G. 2011. Seed viability, germination and seedling growth of canola (*Brassica napus* L.) as influenced by chemical mutagens. *African J Biotch* **10:** 12602-12613.

Feldmann KA, Malmberg RJ and Dean C. 1994. Mutagenesis in *Arabidopsis*. In: Arabidopsis, pp: 137–172. Meyerowitz, E.M., C.R. Somerville (eds.). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA.

Ferrie AMR, Taylor DC, MacKenzie SL, Rakow G, Raney JP and Keller WA. 2008. Microspore mutagenesis of *Brassica* species for fatty acid modifications: a preliminary evaluation. *Plant Breed* **127:** 501-506.

Fowler DB and Stefansson BR. 1972. Effects of the mutagenic agent EMS on the M1 generation of rape *(B. napus*). *Can J Plant Sci* **52:** 53-62.

Hajara NG. 1979. Induced of mutations by chemical mutagens in tall Indica rice. *Indian Agric* **23:** 67-72.

Khalatkar AS and Indurkar HS. 1991. Mutations for double zero *B. juncea. In : Rapeseed in a changing world. Proc GCIRC* **5:** 1549-1554.

Khatri A, Khan IA, Siddiqui MA, Raza S and Shahnizamani G. 2005. Evaluation of high yielding mutants of *B. juncea* cv. S-9 developed through Gamma rays and EMS. *Pak J Bot* **37:** 279-284.

Kumar A, Singh BK, Meena HS, Singh VV, Singh YP and Singh D. 2015. Cytomorphological and molecular characterization of F_1 hybrids between *B. tournefortii* and *B. rapa*. *Cytologia* **80**: 317-326.

Kumar A, Singh BK, Singh VV and Chauhan JS. 2013a. Cytomorphological and molecular evidences of synthesis of interspecific hybrids between *B. rapa* and *B. fruticulosa* through sexual hybridization. *Aust J Crop Sci* **7:** 849-854.

Kumar PKA, Boualem A, Bhattacharya A, Pratikh S, Desai N, Zambelli A, Leon A, Chatterjee M and Bendahmane A. 2013b. SMART – Sunflower mutant population and reverse genetic tool for crop improvement. *BMC Plant Biology* **13:** 1471-2229.

Landge SP and Khalatkar AS. 1995. Early flowering induced mutation in *B. napus* cv. *Westar GCIRC 9th International Rapeseed Congress Cambridge, UK* **3:** 742-744.

Meena HS, Kumar A, Ram B, Singh VV, Meena PD, Singh BK and Singh D. 2015. Combining ability and heterosis for seed yield and its components in Indian mustard (*B. juncea* L.). *J Agr Sci Tech* **17**: 1861-1871.

Meinke DW, Cherry JM, Dean C, Rounsley SD and Koornneef M. 1998. *Arabidopsis thaliana* : a model plant for genome analysis. *Science* **282:** 679–682.

Minocha JL and Arnason TJ.1962. Mutagenic effectiveness of ethyl methane sulphonate in barley. *Nature* **196:** 499.

Okagaki RJ, Neffer MG and Wessler SR. 1991. A deletion common to two independently derived waxy mutations of maize. *Genetics* **127:** 425-431.

Parry MA, Madgwick PJ, Bayon C, Tearall K, Hernandez-Lopez A, Baudo M, Rakszegi M, Hamada W, Al-Yassin A, Ouabbou H, Labhilili M and Phillips AL. 2009. Mutation discovery for crop improvement. *J Exp Bot* **60:** 2817-2825.

Pratap P, Meena PD, Singh BK, Meena HS, Meena SS, Sharma P, Majumdar R and Singh D. 2014. Development and evaluation of Alternaria blight tolerant lines in Indian mustard (*B. juncea*). *J Oilseed Brassica* **5**: 141-148.

Schnurbush T, Mollers C and Becker HC. 2000. A mutant of *B. napus* with increased palmitic acid content. *Plant Breed* **119:** 141-144.

Sestili F, Botticella E, Bedo Z and Phillips A. 2010. Production of novel allelic variation for genes involved in starch biosynthesis through mutagenesis. *Mol Breed* **25:** 145–154.

Singh BD. 2000. Mutations in Crop Improvement. In: Plant Breeding: Principles and Methods. Kalyani Publishers, New Delhi pp 598-631.

Spasibionek S. 2006. New mutants of winter rapeseed (*B. napus* L.) with changed fatty acid composition. *Plant Breed* **125:** 259-267.

Szarejko I and Forster BP. 2007. Doubled haploidy and induced mutation. *Euphytica* **158:** 359-370.

Talebi AB, Talebi AB and Shahrokhifar B. 2012. Ethyl Methane Sulphonate (EMS) induced mutagenesis in Malaysian rice (cv. MR219) for lethal dose determination. *American J Plant Sci* **3:** 1661-1665.