Ethyl Methanesulfonate (EMS)-Mediated Fruit Mutants Of Bitter Gourd (Meghna-2): A Popular Landrace Of West Bengal

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Abstract
Ethyl methanesulfonate (EMS) is a stable and effective chemical mutagen. Bitter Gourd (Meghna-2) seeds were treated by 0.3, 0.5, and 1% EMS for 24 h and 48 h to optimize EMS mutagenesis. Median lethal dose of EMS was obtained at 0.3% EMS treated for 48 h. After treated by 0.3% EMS for 48 h, 250 M1 plants were grown in field for phenotype determination. In comparison to the control, a decline in germination, leaf shape, fruit size, fruit shape, vine length, and emergence was seen in the M1 generation when the concentration of applied EMS was increased. The fertility of M1 bitter gourd was very low and only 24 lines produced seeds after self crossing. Of the mutant 24 lines in the plant growth, five were dwarf, two were tall, four had retarded fruit size, and 13 had abnormal branching and flowering. Only four lines (G 6, G 9, G 14 and G 15) raised from M2 generations of Meghna-2, were screened critically and observed no significant reduction in seed germination and pollen viability but the fruit size was significantly decreased. Interestingly, the short fruit type and round shape of Meghna-2 (G 9) was chosen amongst the four due to farmer's acceptability, which was grown further to confirm the stability.

Keywords: Bitter gourd, Ethyl Methanesulfonate, Meghna-2, Mutagenesis

INTRODUCTION

The vegetable *Momordica charantia* L., Cucurbitaceae, is known variously as bitter gourd, balsam pear, bitter melon, bitter cucumber, and African cucumber (Heiser 1979). The genus includes 59 species distributed widely in Africa and Asia (de Wilde and Duyfjes 2002; Schaefer and Renner, 2010). Although it has many culinary uses, especially in south, southeast and east Asia, it is also grown as an ornamental and is used extensively in folk medicine (Heiser 1979). The fruits are cooked with other vegetables, stuffed, stir-fried, or added in small quantities to beans and soups to provide a slightly bitter flavor. Considerable variation in nutrients, including beta-carotene, vitamin C, B vitamins, folic acid, iron, zinc, calcium, magnesium, phosphorous, and ascorbic acid, has been observed in bitter gourd (Dhillon *et al.* 2016). The fruits are bitter to taste due to the presence of substance called cucurbitacin. Bitter gourd is also reported to use against diseases like paralysis, indigestion and vomiting pain and diabetes (Meir and Yaniv 1985). Fruits and other part of bitter gourd are reported to have cooling, stomachic, appetitising, carminative, antipyrietic, antihelminthic, aphrodisiac and vermifuge properties (Blatter *et. al.* 1935). Various medicinal uses with clinical properties of insulin have been isolated from this species (Baldwa *et al.* 1977). Cucurbit breeders have worked to improve bitter gourd over the years by selecting individual plants from farmers' varieties or landraces, inbreeding those plants to create improved lines or varieties, merging those improved lines or varieties, and identifying commercial hybrids. This strategy was used to boost the likelihood of creating lines with desirable qualities, such as attributes favoured by farmers (earliness, high commercial yield, pest and disease resistance, etc.) and traits favoured by consumers (fruit colour, shape, size, skin pattern, etc.). The use of elite inbred lines repeatedly for hybrid development in order to satisfy the demands of many stakeholders (growers, distributors, retailers, and consumers) has resulted in a narrowing of the crop's genetic base and limited genetic diversity among commercial cultivars (Duvick 2005).

The fundamental for plant survival in nature and agricultural enhancement is genetic diversity. An absence of genetic diversity can make a crop more susceptible to outbreaks of disease and insects (Keneni *et al.* 2012). This is where mutation breeding offers one of the most promising ways to increase genetic variation and improve crops in a short time. For the development of numerous economically significant features in food grain crops, induced mutagenesis has been used to quickly alter the targeted genetic material (Wani *et al.* 2012, Laskar and Khan 2014, Amin *et al.* 2015, Irshad *et al.* 2020) including tomato (Laskar *et al.* 2016, Das *et al.* 2019), okra (Hegazi and Hamildeldin 2010; Asare *et*

al. 2017), cowpea (Kumar and Verma 2011), cauliflower (Hadi and Fuller 2013), garden pea (Aney 2014), and, cucumber (Shah *et al*. 2015). In addition to being used to create genetic variation for crop development, mutagenesis is frequently seen as a highly scholarly endeavour.

Alkylating agents were the first class of chemical mutagens to be discovered when Auerbach and Robson (1946) found the mutagenic effects of mustard gas and related compounds during World War II. Alkylating agents such as mustard gas, methyl-methanesulfonate (MMS), ethyl-methanesulfonate (EMS), and nitrosoguanidine have several effects on DNA. Because of its potency and ease with which it can be used, EMS is the most commonly used chemical mutagen in plants. EMS alkylates guanine bases and leads to mispairing-alkylated G pairs with T instead of C, resulting in primarily G/C to A/T transitions. EMS produces a large number (genome-wide) of non-lethal point mutations, a relatively small mutant population (approximately 10,000) is sufficient to saturate the genome with mutations. In Arabidopsis, point mutation density can be as high as four mutations per Mb (Comai and Henikoff 2003, 2006, Till *et al*. 2003). The offspring (M1) of radiation mutagenesis in bitter gourd also possessed features that are significant commercially and are governed by a single recessive gene (Miniraj *et al*. 1993). High yielding seeds of the landrace MC-103 were used to create the cultivar MDU-1, which was created as a result of gamma radiation treatment (Rajasekharan and Shaninugavelu 1984). Balloch *et al.* (2002), developed a high yield rice species as a result of their 150 Gy application. Recently, Induction of mutation by gamma rays at five different doses was studied in four widely divergent bitter gourd genotypes BG-1346501, Meghna-2, Special Boulder and Selection-1 in Bitter gourd (Dutta *et al.* 2021). However, EMS mutagenesis hasn't been studied much in Bitter gourd. The Meghna-2 bitter gourd EMS mutant population was developed in the current work in order to select mutants with high yields, short fruit sizes, and all Meghna-2 characteristics to meet the farmer's high demand.

MATERIALS AND METHODS

During the spring and summer (February to June) seasons of three consecutive years, field tests were carried out at the Debgiri Agro Products Private Limited Research Farm in Daripukur, North 24 Parganas, West Bengal, India (2019–2021). Over the years, the range of the average minimum and maximum temperatures during the farming seasons was 16 to 44 °C.

Plant Materials

Meghna-2, the landrace of Bitter gourd was employed in the present investigation for its vigorous growth, virus tolerance, suitable for both pandal and soil cultivation. It takes 55-60 days to harvest and the average fruit weight is 130-150 g, fruit is 14-16 cm long, deep green, prominent spines and very bitter in taste.

Determination of Median Lethal Dose

Seed germination percentage in M1 generation was employed to calculate the LD50 dose of the mutagen. One hundred of bitter gourd seeds were soaked in 20 ml of distilled water at low speed shaker for 30min, and then EMS (Sigma, USA) was added to the distilled water at the final concentration of 0.3, 0.5, or 1% (w/v) for 24 h and 48 h at low speed shaker, respectively. The treated seeds were incubated at 28˚C for 15 h after being washed with 3% sodium thiosulfate and distilled water, respectively. Seeds not treated by EMS were incubated as control under the same conditions as EMS-treated seeds. The lethal dose (LD) was calculated as follow:

$$LD\% = (1 - \text{Germination rates of treated seeds/Germination rates of Control}) \times 100$$

Development of mutagenic populations and evaluation

All M1 plants were selfed and seeds were harvested from individual plant to raise M2 generation. For M2 seed production, 250 M1 plants were grown in field (February– July 2019). M1 plants were observed, and their phenotypic data were recorded. After fruit ripening, the mature seeds were harvested, sorted, labeled, air dried, packed, and stored at the seed storage.

Ten plants each from 24 M2 families were grown. The M2 generation was screened critically as it was expected to be the most variable population and all the variants were expected to express themselves. The M2 seeds were sown in well-prepared beds during spring-summer season (February– July 2020).

Mutant screening in the M2 generation

The M2 generation was screened critically based on both qualitative and quantitative characters. All the plants that showed prominent variation in M2 generation for short fruit size characters were selfed to raise M3 generation of single M2 plant progenies. All the selected M3 families comprising at least 30 plants in each line along with the parental bases were raised in plant-to-row plots in M3 generation during February–July 2021.

Statistical Analysis

Experiments were designed in a completely randomized block design and all the experiments were conducted three times. The data were submitted to one-way ANOVA and significant difference among the treatment means was

analyzed by Duncan's multiple-range test (Duncan 1955) using SPSS (Version 15, SPSS Inc. Chicago, USA) at 5% level of significance.

RESULTS AND DISCUSSION

Effects of EMS mutagenesis on M1 plants

Median lethal dose (LD50) is a critical parameter for chemically induced mutagenesis. This value is determined by both mutagen concentration and treatment time and varies in different species and even different cultivars (Ref). The LDs in each EMS treatment were approximately 5, 32, 76, 52, 83 and 85% when 0.3, 0.5, or 1.0% EMS at 24 h and 48 h were used for mutagenesis, respectively (Table 1). When the seeds were treated with 0.3% EMS for 48 h, LD value was 52%, which is very close to LD50. Therefore, the combination of 0.3% EMS and 48 h was used for subsequent mutagenesis. EMS concentration and treatment time showed that EMS significantly ($P < 0.05$) affected the rate of germination. The rate of germination of Meghna-2 seeds in 48 h treatment was higher (48%) in the presence of 0.3% EMS. To justify the results, there are reports where the optimum dosage of EMS for rice, soybean, and tomato is below 1% (Talebi *et al*. 2012, Sikder *et al*. 2013, Arisha *et al*. 2015), but it is also higher (1.5%) for some pepper cultivars (Alcantara *et al*. 1996, Hwang *et al*. 2014). Increased treatment time may allow for seed hydration, which may facilitate the uptake of mutagen and improve the metabolic activity of cells as well as initiation and DNA synthesis in the growing embryo (Natarajan and Shivasankar 1965, Seetharami and Prabhakar 1983), leading to higher germination. After induction by 0.3% EMS for 48 h, the treated seeds (M1) were planted. Only 24 lines out of 250 M1 plants produced M2 seeds. Fertility rate of M1 plants was 27%. Low fertility is the common phenomenon observed in mutant plants by physical and chemical mutagenesis possibly due to severe damage of mutagens to plant genetic materials (Girija *et al*. 2013, Kim *et al*. 2006). The main reason for this low mutation frequency is that most of the mutants bearing multi-mutational events may be lethal in the first generation (Waghmare *et al*. 2001).

Characterization of M2 Mutant Phenotypes

The most conspicuous mutant class observed in the M2 generation was plant growth and fruit size. The mutant phenotypes included in this category were: aberrant plant height (tall or dwarf when compared with wild-type plants), variation in fruit size (short in compared to wild), retarded growth with no visible stem, and abnormal branching as well as flowering. Of the mutant 24 lines in the plant growth, 5 were dwarves, 2 were tall, 4 had retarded fruit size, and 13 had abnormal branching and flowers.

Effect on fruit size

The mutant lines showed decreased length of fruit than the control line (16.66 cm). Different fruit shapes, the conical, oblate and round were noticed, of which former two shapes were present in all the explants tested but the round fruits were observed only G 9. Among the four lines, G 14 (12.22 cm) gave maximum fruit size followed by G 15 (11.43 cm), G 6 (10.87 cm) and the minimum fruit size was found in G 9 (10.56 cm). Fruit length of G 9 was highly reduced as compared to all other Meghna-2 mutant. The short fruited snake gourd mutants were also obtained earlier by Sardar *et al.* (1987). Fruit shape is critical for the market value of a horticultural commodity. Mutants with change in fruit shape can lead to profitable new cultivars according to market demand. Several mutants were characterized with round, oval, cylindrical, and pyramid-shaped fruits. Likewise, effective and a wide range of induced variability were observed in snake gourd mutant lines for short fruit, higher fruit diameter and reduced vine length (Sidhya and Pandit 2015).

Effect on yield

The number of fruit and fruit yield (weight) per plant was significantly influenced. In the present research, the number of fruit per plant was increased but the weight of the fruit was decreased G 9 (Table 2) in comparison to control. Similarly, lower fruit weight in M4 generation of snake gourd has also been also reported by Datta (1994). However, Kumar *et al.* (2002) and Layek *et al.* (2019) reported increased fruit weight in sponge gourd and snake gourd respectively. Mutants with high yield, increased vitamin C (ascorbic acid), antioxidant capacity, enhanced pollen viability and fruit rot resistance were also reported in Solanaceae crop mutation breeding (Tomlekova *et al*. 2009, Öztürk *et al*. 2012), According to Dhillon *et al*. (2018) the 0.15% EMS increased number of pods per plant which may be directly related to yield per plant and it will ultimately increase the total yield.

CONCLUSION

Bitter gourd is an important vegetable crop of several countries in the tropics. Bitter gourd fruit contain bioactive components with many important medicinal properties. In this study, the biological effect of different concentrations of EMS and different environmental conditions on the Bitter Gourd Meghna-2 was determined as well as its sensitivity to mutagen. Isolation of one promising putative mutants with round shaped short size fruit from M3 generation could be utilized for development of commercial hybrid. Future breeding of selected round shaped fruit line and genetic emphases in Bitter Gourd improvement should be placed on the development of nutritious, high-yielding cultivars with superior resistance to major diseases and exceptional fruit quality for both domestic and foreign markets. These efforts should focus on breeding for season and regional adaptation.

FUTURE SCOPE

With the help of EMS mutagenesis, novel mutants with desirable traits such as disease resistance, improved yield, or quality can be generated in bitter gourd. These mutants can be identified through extensive screening and characterization. The identified mutants can be used as a source of genetic variation for breeding new bitter gourd cultivars with improved traits. The development of new cultivars can lead to increased yield, improved quality, and resistance to biotic and abiotic stresses. With the advancements in next-generation sequencing technologies, the genome of bitter gourd can be sequenced to identify the genes responsible for the observed traits. This can lead to a better understanding of the genetic basis of the traits and aid in the development of molecular markers for breeding. With the help of CRISPR-Cas9 gene editing technology, the function of the identified genes can be studied in detail, which can lead to a better understanding of the molecular mechanisms underlying the traits. This can also lead to the development of new biotechnological approaches for crop improvement.

Table 1: Effect of EMS levels and treatment time on the rate of germination, plant fertility, and lethal dose of Bitter Gourd Meghna-2 at 28°C in M1 generation.

EMS conc (%)	Time (h)	Seed number in M1	Germination rate of M1 seeds (%)	Lethal dose (LD%)	Fertile M1 plants (%)
0		100	95	0	100
0.3	24	100	82	18	88
0.5	24	100	68	32	71
1.0	24	100	24	76	23
0.3	48	100	48	52	27
0.5	48	100	17	83	11
1.0	48	100	5	95	8

Table 2: Mean performance of various growth parameter and yield components of Meghna-2 mutant

	Vine Length	Branches Per Vine	Days to First Male Flowering	Days to First Female Flowering	Fruit Length (cm)	Fruit Diameter (cm)	Fruits Per Plant	Fruit Weight (g)	Fruits Yield Per Plant (Kg)
C	4.33	35.45	54.78	61.33	16.66	4.35	18.24	130.45	2.37
G 6	3.25	31.33	55.33	62.25	10.87	4.20	20.45	92.45	1.89
G 9	3.45	35.66	51.45	59.63	10.56	4.33	24.46	90.24	2.20
G 14	3.33	37.00	53.25	61.33	12.22	3.25	24.25	85.66	2.07
G 15	2.85	32.33	53.45	60.00	11.43	4.83	17.45	105.1	1.83
Mean	3.44	34.35	53.65	60.91	12.35	4.29	20.97	100.78	2.07

Figure 1: Bitter Gourd cultivation and characterization of fruits. a. Field cultivation, b. Meghna 2 (control), c. Mutant G 6, d. Mutant G 9, e. Mutant G 14, f. Mutant G 15

Conflict of Interest: The authors should declare that they do not have any conflict of interest.

Author contributions: Conceptualization and designing of the research work (US, DG, MKS); Execution of field/lab experiments and data collection (US, DG, MKS); Analysis of data and interpretation (US, MKS, SK); Preparation of manuscript (US, SK). All the authors approved the final version of the manuscript prior to submission.

REFERENCES

1. Alcantara T.P., Bosland P.W. & Smith D.W. (1996). Ethyl methanesulfonate-induced seed mutagenesis of *Capsicum annuum*. *Journal of Heredity* 87(3), 239-241.
2. Amin R., Laskar R.A. and Khan. S. (2015). Assessment of genetic response and character association for yield and yield components in Lentil (*Lens culinaris* L.) population developed through chemical mutagenesis. *Cogent Food & Agriculture* 1.1, 1000715.
3. Aney A. (2014). Effect of gamma irradiation on yield attributing characters in two varieties of pea (*Pisum sativum* L.). *International Journal of Life Science* 1, 241–247.
4. Arisha M.H., Shah S.N.M., Gong Z.H., Jing H., Li C. & Zhang H.X. (2015). Ethyl methane sulfonate induced mutations in M2 generation and physiological variations in M1 generation of peppers (*Capsicum annuum* L.). *Frontiers in plant science* 6, 399.
5. Asare A.T., Mensah F., Acheampong S., Asare-Bediako E. & Armah J. (2017). Effects of gamma irradiation on agromorphological characteristics of okra (*Abelmoschus esculentus* L. Moench.). *Advances in Agriculture* 2017.
6. Auerbach C. & Robson J.M. (1946). Chemical production of mutations. *Nature* 157(3984), 302-302.
7. Baldwa V.S., Bhandari C.M., Pangaria A. & Goyal R.K. (1977). Clinical trial in patients with diabetes mellitus of an insulin like compound obtained from plant source uppasala. *Journal of Medical Science* 82, 85-89.
8. Balloch A.W., Soomro A.M., Javed M.A., Bughio H.R., Bughio M.S., Mohammed T. & Mastoi N.N. (2002). Development of High Yielding Rice Mutant Variety Through Gamma Rays Irradiation. *The Nucleus* 39 (3- 4), 227-231.
9. Blatter E., Caius J.F. & Mahaskar K.S. (1935). Indian medicinal plants. [2nd Edtn] M/S Bishan Singh, Dehradun. p., 1130-1132.
10. Comai L. & Henikoff S. (2006). TILLING: practical single-nucleotide mutation discovery. *The Plant Journal* 45(4):684-694.
11. Das I., Hazra P., Longjam M., Bhattacharjee T., Maurya P.K., Banerjee S. & Chattopadhyay A. (2019). Characterization of induced mutants and their hybrids of tomato (*Solanum lycopersicum* L.) for growth, yield and fruit quality traits to explore the feasibility in future breeding. *Genetic Resources and Crop Evolution* 66, 1421–1441.
12. Datta, S.K. (1994). Induction and analysis of short thick fruit mutant in *Trichosanthes anguina* L. Indian *Journal of Genetics and Plant breeding* 54(1), 13-17.
13. De Wilde, WJJO & Duyfjes B.E.E. (2002) Synopsis of *Momordica* (Cucurbitaceae) in SE-Asia and Malesia. *Botanicheskii Zhurnal (St. Petersburg)*, 87, 132-148.
14. Dhillon N.P., Sanguansil S., Srimat S., Cheng H., Lin C., Ramasamy S., Kenyon L., Schafleitner R., Yang R. & Hanson P. (2016). Status of cucurbit breeding at AVRDC-the World Vegetable Center. In *Cucurbitaceae 2016, XIth Eucarpia.* Cucurbitaceae 2016 Organizing Committee. *Meeting on Cucurbit Genetics & Breeding*, July 24-28, 2016, Warsaw, Poland. p., 21-25.
15. Dhillon T.S., Dhall R.K. & Gill B.S. (2018). Effect of mutagen-ethyl methane sulphonate on yield increasing parameters of French bean (*Phaseolus vulgaris* L.). *Genetika* 50(1), 199-207.
16. Duncan D.B. (1955) Multiple range and multiple F test. *Biometrics* 11, 1–42.
17. Dutta S., Hazra P., Saha S., Acharya B., Bhattacharjee T., Maurya P.K., Banerjee S., Chakraborty I. & Chattopadhyay A. (2021). Applied mutagenesis could improve economically important traits in bitter gourd (*Momordica charantia* L.). *Journal of Genetics* 100(2), 1-13.
18. Duvick, D.N. (2005). The contribution of breeding to yield advances in maize (*Zea mays* L.). *Advances in agronomy* 86, 83-145.
19. Girija M., Dhanavel D. & Gnanamurthy S. (2013). Gamma Rays and EMS Induced Flower Color and Seed Mutants in Cowpea (*Vigna unguiculata* L. Walp). *Advances inApplied Science Research* 4, 134-139.
20. Hadi F.. & Fuller M.P. (2013). Chemically induced mutants of *Brassica oleracea* var. botrytis maintained stable resistance to drought and salt stress after regeneration and micropropagation. *American Journal of Plant Sciences* 4, 498–507.
21. Hegazi A.Z. & Hamideldin N. (2010). The effect of gamma irradiation on enhancement of growth and seed yield of okra [*Abelmoschus esculentus* (L.) Monech] and associated molecular changes. *Journal of Horticulture and Forestry* 2, 38–51.
22. Heiser C.B. (1979). The gourd book. University of Oklahoma Press, Norman, OK.
23. Henikoff S. & Comai L. (2003). Single-nucleotide mutations for plant functional genomics. *Annual Review of Plant Biology* 54(1), 375-401.
24. Hwang D., Jeong H..J., Kwon J.K., Kim H.., Kang S.Y. & Kang B.C. (2014). Phenotypic variants among ethyl methanesulfonate M 2 mutant lines in *Capsicum annuum*. *Plant Genetic Resources* 12, S141–S145.

25. Irshad A., Guo H., Zhang S., & Liu L. (2020). TILLING in cereal crops for allele expansion and mutation detection by using modern sequencing technologies. *Agronomy* 10(3), 405.
26. Kim Y., Schumaker K.S. & Zhu J.K. (2006). EMS Mutagenesis of *Arabidopsis*. *Methodsin Molecular Biology* 323, 101-103.
27. Kumar G. & Verma S. (2011) Comparative effect of individual and sequential treatment of gamma rays and sodium azide in *Vigna unguiculata*. *Chromosome Botany* 6, 33–36.
28. Kumar S., Kumar S., Singh B., Virendra Jatav A.L. & Kumar S. (2002). Influence of gamma radiation of seed on growth, sex expression and yield in sponge gourd (*Luffa cylindrica* L.). *Plant archives* 2(2), 25-28.
29. Laskar R.A. & Khan S. (2014). Mutagenic effects of MH and MMS on induction of variability in broad bean (*Vicia faba* L.). *Annual Research & Review in Biology* 4, 1129–1140.
30. Laskar R.A., Chaudhary C., Khan S. & Chandra A. (2016). Induction of mutagenized tomato populations for investigation on agronomic traits and mutant phenotyping. *Journal of the Saudi society of agricultural sciences* 17, 51–60.
31. Layek S., Pandit M.K., Kumar S., Das A. & Upadhyay A. (2019). Qualitative and Quantitative Characterization of Snake Gourd (*Trichosanthes anguina* L.) Mutant Lines. *International Journal of Current Microbiology and Applied Sciences* 8(8), 1797-1803.
32. Meir P. & Yaniv Z. (1985) An *in vitro* study on effect of (*Momordica charantia L*) on glucose uptake and glucose metabolism in rats. *Plants Medica* 1, 12-16
33. Natarajan A. & Shivasankar G. (1965). Studies on modification of mutation response of barley seeds to ethyl methanesulfonate. *Z. Vererbungsl.* 96, 13-21.
34. Öztürk S., Kantoğlu Ö., Özmen D., Iç E. & Kantoğlu K.Y. (2012). Phytonutrient analysis of gamma rays mutated tomato. In: International Conference Nuclear Science and its Application Abstracts Book, September 25-28, 2012. Samarkand, Uzbekistan. p., 398-399.
35. Rajasekharan KR & Shanmngavelu K G (1984). MDU-1 bitter gourd. South Indian Hort. 32, 47.
36. Sardar A.K. & Mukherjee K.K. (1987). Analysis of two short-fruit mutants of *Trichosanthes anguina* L. *Indian Journal of Genetics and Plant breeding* 47(3), 257- 260.
37. Schaefer H. & Renner S.S. (2010). A three-genome phylogeny of *Momordica* (Cucurbitaceae) suggests seven returns from dioecy to monoecy and recent long-distance dispersal to Asia. *Molecular phylogenetics and evolution* 54(2), 553-560.
38. Seetharami Reddi T.V. & Prabhakar G. (1983). Azide induced chlorophyll mutants in grain *Sorghum* varieties. *Theor. Appl. Genet.* 64, 147-149.
39. Shah S.N.M., Gongi Z.H., Arisha M.H., Khan A. and Tian S.L. (2015). Effect of ethyl methyl sulfonate concentration and different treatment conditions on germination and seedling growth of the cucumber cultivar Chinese long (9930). *Genetics and Molecular Research* 14, 2440–2449.
40. Sidhya P. & Pandit M.K. (2015). Mutagenic effectiveness and efficiency of gamma rays in snake gourd (*Trichosanthes anguina* L.). *Journal of Applied and Natural Science*. 7(2), 649 – 651.
41. Sikder S., Biswas P., Hazra P., Akhtar S., Chattopadhyay A., Badigannavar A.M. & D'Souza S.F. (2013). Induction of mutation in tomato (*Solanum lycopersicum* L.) by gamma irradiation and EMS. *Indian Journal Genetics and Plant Breeding* 73, 392–399.
42. Talebi A.B., Talebi A.B. & Shahrokhifar B. (2012). Ethyl methane sulphonate (EMS) induced mutagenesis in Malaysian rice (cv. MR219) for lethal dose determination. *American Journal of Plant Sciences* 3, 1661–1665.
43. Till B.J., Reynolds S.H., Greene E.A., Codomo C.A., Enns L.C., Johnson J.E., Burtner C., Odden A.R., Young K., Taylor N.E. & Henikoff J.G. (2003). Large-scale discovery of induced point mutations with high-throughput TILLING. *Genome research* 13(3), 524-530.
44. Tomlekova N.B., Timina O.O. & Timin O.Y. (2009). Achievements and perspectives of sweet pepper breeding towards high beta-carotene. In: Krasteva, L. and Panayotov, N. (eds.) IV Balkan Symposium on Vegetables and Potatoes. *Acta Horticultura* 830, 205-212.
45. Waghmare V.N. & Mehra R.B. (2001). Induced Chlorophyll Mutations, MutagenicEffectiveness and Efficiency in *Lathyrus sativus* L. *Indian Journal of Genetics and Plant Breeding* 61, 53-56.
46. Wani R., Khan S. & Kozger I. (2012). Genetic enhancement of mungbean (*Vigna radiata* (L.) Wilczek) through induced mutagenesis. *Crop Research* 43, 189–193.