Article Type: Research Article
J Name: Modern Phytomorphology
Short name: MP
ISSN: ISSN 2226-3063/eISSN 2227-9555
Year: 2022
Volume: 16
Page numbers: 62-67
DOI: 10.5281/zenodo.200121
(10.5281/zenodo.Year-Volume-PDFNo.)
Short Title: Effect of Ethyl Methyl Sulfonate on Cyto-Morphological variations in *Corchorus capsularis L.*

Research Article

Effect of Ethyl Methyl Sulfonate on Cyto-Morphological variations in *Corchorus capsularis L.*

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Received: 26.05.2022, Manuscript No.: MP-22-68767 | Editor Assigned: 6.06.2022, Pre-QC No. MP-22-68767 (PQ) | **Reviewed**: 12.06.2022, QC No. MP-22- 68767 (Q) | **Revised**: 24.06.2022, Manuscript No. MP-22-68767 (R) | **Accepted**: 19.07.2022 | **Published**: 30.07.2022.

Abstract

Dry seeds of *Corchorus capsularis* L. were treated with three doses of Ethyl methyl sulfonate viz. 0.1%, 0.3% and 0.5%. In a laboratory test, AMI% was decreased and TAB% was increased with increases in the concentration of mutagen. Similarly, germination percentage and survival were also negatively affected by an increase in concentration during the field study. In this experiment, four types of chlorophyll mutation viz. maculata, chlorina, xantha, and aurea were observed xentha and aurea were observed in most of the treated sets, whereas. meculata and chlorina observed only in lower doses viz. 0.1% and 0.3%. Based on the findings during experiment, a negative correlation was obtained between AMI, root length, shoot length, chlorophyll mutants, and concentration of EMS. Results show an increment in chromosomal anomaly at higher concentrations and a wide range of abnormalities were recorded i.e., stickiness, scattering, unorientation, bridges, etc. The application of EMS was affected survival considerably than the germination%.

Keywords: *EMS, Corchorus capsularis L., Mutagenesis, Active Mitotic Index*

Introduction

Climate change is a very important and essential subject at the current time. In recent times there is frequent use of polythene and synthetic fibers cause severe damage to the environment and the hazardous effect of these products causes various diseases in human beings and livestock. Due to this reason, there is a crucial requirement to make our environments and our surroundings pollution-free. To achieve this goal there is the requirement to increase the use of natural products which can be easily recycled by nature. Natural fibres have attracted the attention worldwide recently because it is eco-friendly, renewable, and available in abundance amount. Its importance also increased after the announcement of the UN that year 2009 was the 'International Year of Natural Fibres' (Bhandari et al., 2018).

Corchorus capsuaris L. generally known as jute is a natural phloem fiber crop that has ample economic importance after cotton (*Gossypium hirsutum* L. and *Gossypium arboretum* L.) as it is completely environment friendly, recyclable, and biodegradable fiber constituting cellulose and lignin (Mir et al., 2008). About 100 species of Corchorus worldwide mainly two species are cultivated commercially i.e. *Corchorus capsularis, Corchorus olitorious* (Hossain et al., 2002). Apart from its conventional uses, it gathered importance due to its diversified value-added products. Jute incorporates about 50% of the total production of bast fibre globally. For the textile and paper business jute is a very valuable constituent as raw material along with being a sustainable source (Wazmi et al. 2007).

Since jute fibre blends with viscose, polyester, and cotton to form diverse textile and garments goods, which are sustainable and renewable. Several qualities of jute-like, bundle strength, lignin content, and fineness of fibre attracts breeders. It is an option for elimination of synthetic fibres, its demand increases in international markets with improved quality of fibre and yield. To achieve such criteria breeders has to develop different variety of plants with improvement genetic traits. Any breeding programmes mainly depends on availability of variation in genome, which creates possibilities for breeders to select plants with superior and improved characters of interest for both agriculturists along with consumers. Jute has narrow genetic makeup (chromosome number 2n=14)

as well as self-pollinating crop, which make it difficult to create mutation naturally. For broadening of gene pool within species through generation of mutation in shorter time period is attained via induced mutagenesis (Mandal & Datta 2014).

Experimentally induced mutation has contributed significantly to global agriculture by producing more than 3000 mutants with desirable qualitative and quantitative traits in about 175 crop species (Kharkwal 2012). Induction of mutation may attain via physical and chemical mutagens or their combination. In present experimental work EMS, as alkylating agent used for mutagenesis (Kozgar et al., 2011), causes ethylation of bases in DNA throughout the modification of oxygen present in the nucleotide bases as well as in DNA phosphate groups (Kumar & Pandey 2018). Present investigation describes the variation in treated replicates along with control sets including various traits like morphometric, cytogenetical (mitosis of root apical meristem) and leaf variants under uniform environmental conditions.

Material and Methodology

Plant material

Inbred seeds of Corchorus capsularis L. var. JRC 698 was procured from Central Research Institute for Jute and Allied Fibres, West Bengal, India.

Treatment and climatic condition

The present experiment has been performed in the area of Roxburgh Botanical Garden, Department of Botany, University of Allahabad, Prayagraj, U.P., India, during the Kharif season. The exact experimental location is 25°27"43.01'N, 81°51"10.42'E. Prayagraj is situated 98 m above mean sea level. Prayagraj is in the sub-tropical climatic zone; the average rainfall is 1027 mm and the relative humidity is 59%. Temperature in mid-march at the time of sowing were 28-30°C. Inbred and healthy seeds of Jute were soaked in distilled water for 24 hours to increase the permeability for further reaction. After that, the seeds were treated with different concentrations of EMS solution (0.1%, 0.3%, and 0.5%) prepared in pottasium phosphate buffer at pH 7 for 3 hours. Along with the treatment, control sets were also maintained by seeds soaked in distilled water. After treatment, the seeds were washed 3-4 times under tap water to remove the excess chemical. For mitotic analysis, treated sets after the recovery period were placed in germinator at 30 to 35°C in petri plates with filter papers. After 3-4 days germinated seeds having radicle lengths of 3-5 mm sizes were fixed in carnoy's fixative for 24 hours and then transferred in 90% alcohol. Some seeds were sown in earthen pots along with control in a random block design with 3 replications for morphological study observation.

Cytological analysis

For squash preparation staining was done in 2% Acetocarmine stain for 30-60 minutes. Slides were prepared and observed under Nikon eclipse E200 with MICAPS- microview software.

Mitotic formula

Mitotic index was calculated according to Edgar (1961) and Balog (1982):

$$\text{ActiveMitoticindex} = \frac{\text{Total no. of dividing cells}}{\text{Total no. of cell observed}} \times 100$$

$$\text{Total Abnormality percentage} = \frac{\text{No. of Abnormal cells}}{\text{Total no. of cell observed}} \times 100$$

Morphological analysis

= No. of Abnormal cells Total no. of cell observed×100

Germination percentage was recorded after 7 days of sowing and survivability of plants was calculated after 14 days and the data were recorded in triplicates.

Data analysis

All results were presented statistically and tested with one way ANOVA (Analysis of Variance) followed by Duncan's Multiple Test Range (DMRT) ($P \leq 0.05$) significance level with the use of SPSS software. Graphical representation was done using Sigma Plot 10.

Results

The cytological estimation of EMS shows a noteworthy impact on the cytology of root meristems of jute. It can causes significant changes on the mitotic activity and chromosomal morphology of plant. Tab. 1 represent complete data of AMI% and TAB%. The root tip are directly exposed to the mutagenic treatments, the effects of known concentrations can be studied. A highly significant decrease in AMI(%)compared to the control was evident at all doses. Data observed from the treated sets of EMS seeds on different concentrations at 3 hours and 5 hours along with control set was depicted comparatively in Tab. 1. A highly significant decrease in AMI (%) compared to the control was evident at all doses. The decrement rate was very higher in case of 5h EMS

treatment duration. In the 3h and 5h of periods, the AMI percentage in controls were 10.56 ± 0.6% and 9.41 ± 0.16%, respectively. AMI declined from 10.56 ± 0.6 % (control) to 9.56 ± 0.7 (0.1%) and up to 7.89 ± 07 (0.5%) in 3h treatment whereas in 5h treatment set the reduction was up to 6.56 ± 0.28 (0.5%). However, in case of lower doses, EMS is not much mito-inhibitory but the rate of decline is accordingly in a dose dependent manner. Whereas TAB(%) increases in dose dependent manner, thus AMI and TAB percentages show inverse relationship (Fig 1). Percentage of various chromosomal abnormalities induced by EMS in the abnormal cells of root meristem of jute plant is documented in Tab. 1. EMS treatment causes disturbance in cell division which leads to imposing several abnormalities in both the stages (metaphase and anaphase). These aberrations are directing the increment in Total abnormality percentage, which is related to the toxicity of EMS concentration.

The comparative analysis trend of TAB% was depicted in Tab. 1. TAB% were increased from 3.37 ± 0.02 (0.1%) to 5.42 ± 0.59 (0.5%) in 3 hours treatment whereas the highest value of TAB% was recorded in 5 hours treatment set of 0.5% (5.44 ± 0.21) concentration of EMS.

The abnormalities reported in this experiment are stickiness, scattering, precocious movement, bridge formation, unorientation, and some others represented in (Fig 1). Stickiness was found to be more prominent at both stages of divisions (metaphase and anaphase) (Fig 2).

For further study, seeds were sown in pots and when the seedlings were 14 days older, the morphological data was collected which includes germination, survival (after 7 days) root length and shoot length (after 14 days). In present experiment, germination % was decrease as doses were escalated in 3h and 5h treatment. Highest germination% was recorded 96.66% and 93.33% in control (3 and 5 h respectively) which decreases 83.33% at 0.1% concentration to 63.33% at 0.1% and from 76.66% at 0.1% concentration to 56.66% at 0.5% concentration at both time periods (3 h and 5 h) .The comparative trend of data was represented in (Fig. 3). Survival% also shows similar trend and value recorded were 90.00% and 86.66% in control set and 76.66% (0.1%) to 56.66% (0.5%) and 70.00% (0.1%) to 46.66% (0.5%) at 3h and 5h treatment respectively.

Effect of treatments at 3h and 5h both, in germination and survival leads an adverse effect on root length and shoot length in contrast with control sets and data was shown in (Fig. 4).Value found to be lowest was 0.6 cm (at 0.5% in 5h) and 2.5 cm (at 0.5% in 3h) whereas maximum value recorded wein re 2.control 7 cm and 2.2 cm (in 3h and 5h).

Gradual decrease was also recorded in shoot length similar to root length.

The maximum shoot length value recorded was 2.8 cm and cm in control sets and the minimum value recorded was 1.9 cm and 0.9 cm in 0.5% treatment sets at both periods.

Table 1. Cytological abnormalities induced by EMS in *Corchorus capsularis L.*

Treatment	Con c.	AMI (%)	Metaphasic abnormality (%)					Anaphasic abnormality (%)					OTH (%)	TAB (%)
			SC	ST	PM	UN	LP	SC	ST	UN	BR	LG		
EMS	Control	10.56 ± 06	-	-	-	-	-	-	-	-	-	-	-	-
3 hours	0.10 %	9.56 ± 0.07	0.41 ± 0.2	0.69 ± 0.16	0.14 ± 0.14	0.39 ± 0.22	-	0.41 ± 0.02	0.41 ± 0.02	0.41 ± 0.02	0.14 ± 0.14	-	0.39 ± 0.22	3.37 ± 0.02
	0.30 %	8.34 ± 0.06	0.45 ± 0.04	0.90 ± 0.08	0.13 ± 0.13	0.45 ± 0.04	0.45 ± 0.04	0.31 ± 0.16	0.90 ± 0.08	0.32 ± 0.16	0.14 ± 0.14	0.31 ± 0.16	0.50 ± 0.31	4.66 ± 0.31
	0.50 %	7.89 ± 0.7	0.46 ± 0.03	0.91 ± 0.07	0.33 ± 0.16	0.45 ± 0.28	0.46 ± 0.03	0.46 ± 0.03	0.91 ± 0.07	0.30 ± 0.15	0.46 ± 0.03	0.16 ± 0.16	0.78 ± 0.16	5.42 ± 0.59
EMS	Control	9.41 ± 0.16	-	-	-	-	-	-	-	-	-	-	-	-
5 hours	0.10 %	8.64 ± 0.20	0.14 ± 0.14	0.69 ± 0.16	0.27 ± 0.13	0.53 ± 0.11	-	0.41 ± 0.02	0.28 ± 0.14	0.27 ± 0.13	0.27 ± 0.13	0.14 ± 0.14	0.41 ± 0.02	3.57 ± 0.08
	0.30 %	7.34 ± 0.17	0.45 ± 0.04	1.04 ± 0.10	0.45 ± 0.04	0.28 ± 0.14	0.31 ± 0.16	0.14 ± 0.14	0.73 ± 0.10	0.31 ± 0.16	0.28 ± 0.14	0.14 ± 0.14	0.63 ± 0.21	4.75 ± 0.10
	0.50 %	6.56 ± 0.28	0.29 ± 0.15	0.75 ± 0.14	0.46 ± 0.03	0.30 ± 0.15	0.16 ± 0.16	0.46 ± 0.03	0.91 ± 0.07	0.33 ± 0.33	0.46 ± 0.03	0.46 ± 0.03	0.88 ± 0.20	5.44 ± 0.21

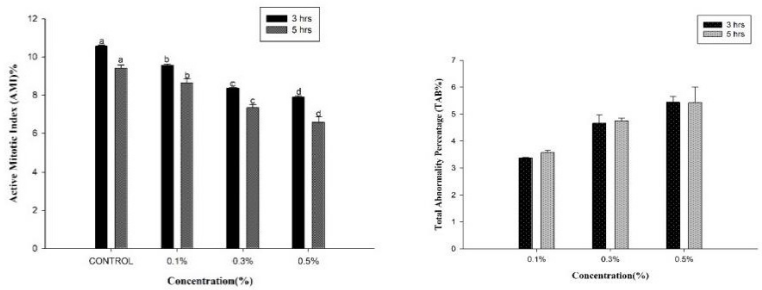

Figure 1. Effect of EMS treatment on AMI% and TAB% in root meristems of Corchorus capsularis L.

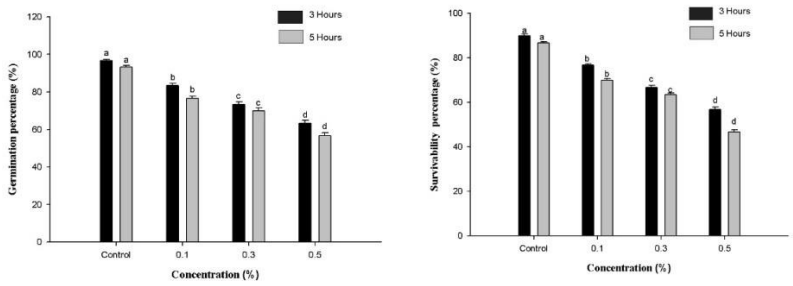

Figure 2. Effect of EMS treatment on germination percentage and survival percentage in Corchorus capsularis L.

Figure 3. Effect of EMS (3h, 5h) treatment on root and shoot length in Corchorus capsularis L.

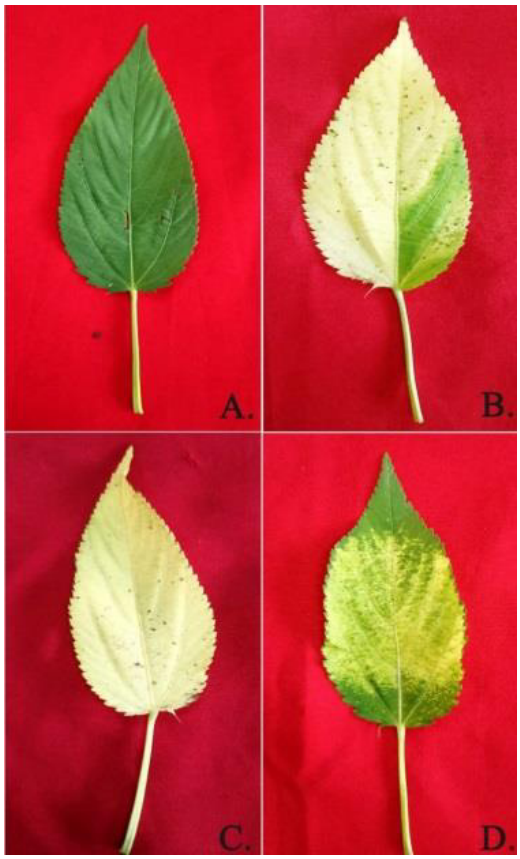

Figure 4. Showing chlorophyll variation (A- Normal: B- Semi-xantha; C-Aurea and D-Meculata) induced by EMS treatment in *Corchorus capsularis L.*

Scattering of chromosome at metaphase, D. Unorientation at metaphase, E. Precocious movement at metaphase, F. C-metaphse G. Early anaphase H. Forward movement at Anaphase I. Multiple bridge formation at Anaphase (Scale bar: Length - 10 µm)

Discussion

Induced mutagenesis is used oftentimes as it creates possibilities for the selection of better characters. Several researchers reported that mutations caused by EMS are distributed across the genome randomly (Greene et al. 2003; Till et al., 2003). EMS as mutagen creates variation and shows mito-depressive activity and also makes changes in morphological parameters.

These changes at the cytological level are clearly assayed by observing the Active mitotic indices and total abnormality percentage of root meristem cells. A decrease in AMI % and increment in TAB% is reported in a dose-dependent manner. The

decline in AMI% may be due to loss of defence mechanism by destruction in proteins involved in DNA synthesis, phase transition, etc. and these results were also found by (Liu et al., 2015).

Stickiness is the foremost aberration among all the anomalies reported. It gives a hint that EMS or chemical mutagen creates variation in an arrangement at the chromosomal level. The chromosomal stickiness could also be observed at high frequency owing to the disturbance in nucleic acid metabolism of the cell (Chidambaram et al., 2006). Kumar and Singh (2007), also found stickiness as a chromosomal aberration in EMS treated plants of Hordeum vulgare L. Such kind of observation was also shown in pulses crop i.e.*Glycine max L.* (Kumar & Rai 2007).

Due to stickiness, chromosomes were unable to move towards the polar region and form connections which lead to the development of bridge at anaphase and also may be due to condensation of proteins present in the nucleus (Khanna & Sharma 2013) (Kumar and rai 2007), (Kumar and Srivastava 2017).

Breakage of chromosomes and dysfunctioning of proteins (spindle fibers) result in precocious movement (Kumar and Rai, 2007). An orientation and scattering of chromosomesare reported at metaphase and anaphase which was as a result of a disturbance in the proteinaceous material of the cytoplasm. Additionally discontinuation of chiasma terminalization shifts toward the formation of laggard formation at anaphase (Verma et al., 2012).

Germination and survivability both the parameters are tremendously decreases with increase in treatment in both the time periods (3h and 5h). Reduction in germination percentage as well as survival might be due to chromosomal damage in meristamatic cells which resulted in retard growth of plant. The mutagenic treatments also delayed the germination process. (Rai & kumar 2007) also suggested that hindrance in initiation of metabolism dependents germination, as a result mitotic activity is consistently delayed, seedling growth, ATP and DNA synthesis leads to reduction in survivability also.

Similar reporting was also found by several authors in reference to gamma irradiation that it induces a reduction in seedling development due to cellular proficiency in DNA repair, even if it is deficient in the ability to arrest in response to damage which leads to high instability in the genome (Preuss & Britt 2013, 2003; Manova and Gruszka, 2015), as well as metabolic disorders (oxidation, rupture of covalent bonds and formation of free radicals) in seeds (Alpen 1998; Stajner et al. 2007; Shikazono et al. 2009; Kiong et al. 2008).

Different kind of abnormality caused by chemical mutagen, leads to create variation in leaves i.e. chlorophyll mutation. Chlorophyll mutations are one of the most reliable indices for evaluating the genetic effects of mutagenic treatments (Kolar et al 2011). Four different types of chlorophyll mutants (maculata, chlorina, semi- xantha, aurea) were recorded in the field. The control had green or light green leaves whereas, maculata are characterized by yellow or whitish dots on leaves. Aurea, which is reported in higher concentration had golden yellow coloured leaves and semi- xantha had pale yellow coloured leaves, these mutants could not survive more than a few days due to block in chlorophyll synthesis (Blixt 1961).

Many researchers have reported different chlorophyll mutations after EMS treatments, such as (kolar et al. 2011) lentil (Solanki 2005), and *Lathyrus sativus* (Ramezani et al. 2014).

Conclusion

From present investigation was concluded that EMS causes negative impact on morphological and cytological behavior of the plants. Mutagen causes point mutation leads to create variability in chromosomal level, higher doses cause severe damage to plants and lower doses are less toxic to plant which may responsible for chlorophyll variation in leaves. Above findings emphasize that least concentration of EMS create promising mutations with less lethality in further upcoming generation and could be utilized in Future practices regarding *Corchorus capsularis L.*

Aknowledgement

Authors shows gratitude to CRIJAF, Kolkatta, West Bengal, India for providing inbred seeds of *Corchorus capsularis L.* The financial support from CSIR-UGC are gratefully acknowledged.

References

Alpen E.L. (1998). Radiation chemistry radiation biophysics. *Elsevier* 104–131.

Balog C. (1982). The mitotic index in diploid and triploid Allium roots. *Cytologia* **47**:689-697.

Bhandari H. R., Bera A., Kar C. S., Biswas S. (2018). Stability assessment of jute seed production system in lower Gangetic plains of India. *Ind Crops Prod* **125**: 505-510.

Blixt S. (1961). Quantitative studies of induced mutations in peas. V. Chlorophyll mutations. *Agr Hort Genet* **19**: 1-402.

Chidambaram A., Murugan L., Sankar A., Ganesh K., Sundaramoorthy P. (2006). Effect of Chromium on growth and cell division of blackgram (*Vigna mungo* (L.) Hepper.). *Plant Arch* **6**: 763-766.

Edgar E. (1961). Fluctuations in mitotic index in the shoot apex of Lonicera nitida.

Greene E.A., Codomo C.A., Taylor N.E., Henikoff J.G. (2003). Till, B.J., Reynolds, S.H., Enns, L.C., Burtner, C., Johnson, J.E., Odden, A.R. andComai, L. 2003. Spectrum of chemically induced mutations from a large-scale reverse-genetic screen in Arabidopsis. *Genetics* **164**: 731-740.

Hossain M.B., Haque S., Haseena K. (2002). DNA Fingerprinting of jute germplasm by RAPD. *J Biochem Mol Biol* **35**: 414–419. Google Scholar Cross Ref

Kharkwal M.C. (2012). Impact of mutation breeding in global agriculture.

Kiong A., Lai A., Hussein S., Harun A. (2008). Physiological responses of orthosiphon stamineus plantles to gamma irradiation. *Am Eurasian J Sustain Agric* **2**: 135–149.

Kolar F., Pawar N., Dixit G. (2011). Induced chlorophyll mutations in *Delphinium malabaricum* (Huth) Munz. *Appl Hortic* **13**:18-24

Kozgar M. I., Goyal S., Khan S. (2011). EMS induced mutational variability in Vigna *radiata* and Vigna *mungo*. *Res J Bot* **6**: 31

Kumar G & Rai P (2007). Genotoxic potential of mercury and cadmium in soyabean. *Turk Journ Biol* **31**: 13-15.

Kumar G., Srivastava A., Singh R. (2020). Impact of nanoparticles on genetic integrity of Buckwheat *(Fagopyrum esculentum* Moench). *Jordan J'Biol Sci* **13**: 13-17.

Liu F., Chen H., Han R. (2015). Different doses of the enhanced UV-B radiation effects on wheat somatic cell division. *Cell Bio* **4**: 30.

Mandal A. & Datta A. K. (2014). New Plant Type Mutants in Jute (*Corchorus Olitorius* L.). *Indian Sci Technol* **7**: 543.

Manova V. & Gruszka D. (2015). DNA damage and repair in plants–from models to crops. *Front plant Sci* **6** 885.

Mir R. R., Rustgi S., Sharma S., Singh R., Goyal A., Kumar J., Gaur A. , Tyagi A. K., Khan H., Sinha M. K., Balyan, H. S., Gupta P. K. (2008). A preliminary genetic analysis of fiber traits and the use of new genomic SSRs for genetic diversity in jute. *Euphytica* **161**:413–427.

Preuss S. B. & Britt A. B. (2003). A DNA-damage-induced cell cycle checkpoint in Arabidopsis. Genetics, **164**: 323-334.

Rai P. (2007). EMS induced karyomorphological variations in maize (Zea mays L.) inbreds. *Turk J Biol* **31**: 187-195.

Ramezani P., Siavoshi M., More A. D., Ebrahimi M., Dastan S. (2017). Gamma Rays and EMS Induced Flower Color Mutation in Grasspea (*Lathyrus sativus* Linn.). *J Agric Sci* **23**.

Shikazono N., Noguchi M., Fujii, K., Urushibara A., Yokoya, A. (2009). The yield, processing, and biological consequences of clustered DNA" damage induced by ionizing radiation. *J Radiat Res* **50**: 27–36.

Solanki I. S. (2005). Isolation of macromutations and mutagenic effectiveness and efficiency in lentil (*Lens culinaris* Medik.). *Indian J Genet Plant 'Breed* **65**: 264-268.

Stajner D., Milosevic M., Popovi´c B. (2007). Irradiation effects on phenolic content, lipid and protein oxidation and scavenger ability of soybean seeds. *Int J Mol Sci* **8**: 618–627 *Plant Tissue Cult Biotechnol 17: 173-182.*

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. (2003). Large-scale discoveryof induced point mutations with high-throughput TILLING. *Genome Research* **13**: 524–530.

Wazni M. W., Islam, A. S., Taliaferro J. M., Anwar N., Sathasivan K. (2007). Novel ESTs from a jute (*Corchorus olitorius* L.) cDNA library.