



Plant Archives

Journal homepage: <http://www.plantarchives.org>DOI Url : <https://doi.org/10.51470/PLANTARCHIVES.2025.v25.no.2.115>

STUDIES ON MUTAGENESIS IN GARLIC USING GAMMA RAYS TO DETERMINE LETHAL DOSE (LD 50) AND INDUCING MUTATION

Swagata Mondal^{1*}, Abujafar Oliyar Rahaman¹, Manas Kumar Pandit¹ and Subham Ghosh²¹Department of Vegetable Science, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, 741252, India.²Division of Agricultural Bioinformatics, The Graduate School, Indian Agricultural Research Institute, New Delhi-110012, India.*Corresponding author E-mail: swagatamandal96@gmail.com

(Date of Receiving-04-07-2025; Date of Acceptance-19-09-2025)

ABSTRACT

The experiment aimed to assess the effects of varying gamma radiation doses on seedling characteristics and determine the optimal radiation doses for garlic. Uniform size bulbs of one popular garlic variety namely *Balagarh local* harvested during *rabi* 2022 irradiated with gamma rays at 0, 5, 10, 15 and 20, 25Gy at the gamma chamber (GC-5000) of the Regional Nuclear Agricultural Research Centre, BCKV, Mohanpur, Nadia, West Bengal, India. The study reveals that increased radiosensitivity in garlic leads to a higher percentage of mutants, as over 50% of mortality can be achieved with high doses of radiation. The optimal dosage for gamma radiation was determined to be 12.73Gy, with GR50 values for germination percentage at 18.4 Gy. The study found that higher doses of gamma radiation were detrimental to plant vigor and germination 10 Gy to 15 Gy. The shoot length and root length averages of different treatment levels were significantly different, with GR50 values for root length, leaf count, fresh weight, and dry weight varying from 6.04Gy to 24Gy. LD50 and GR50 doses are recommended for large-scale mutation breeding experiments due to their potential to increase the frequency of desired mutations. Gamma irradiation can cause unwanted mutations, while lower doses have negligible effects on the genome. Traditional breeding methods for garlic are challenging due to its vegetative mode of reproduction and long generation times. GR50, a 50% reduction in parameters, can be used to achieve desired mutations without causing plant mortality.

Key words: LD50, GR50, garlic, gamma radiation, radiosensitivity, optimum doses.

Introduction

Garlic (*Allium sativum* L.) is one of those herbs that has been studied extensively over many years and has been used to treat infectious diseases for ages (Onyeagba *et al.*, 2004). Alliums are one of the most often consumed vegetable species in the world. There are over 600 species in the genus *Allium*, which are found in Europe, North America, North Africa, and Asia. The number of species in the genus has continuously increased over time as botanists find new species all over the world. The plants of the *Allium* genus are almost exclusively herbaceous, perennials, and usually form bulbs. However, some species produce thicker rhizomes. Garlic (*Allium sativum*) and onion (*Allium cepa*) are the most widely consumed members of the genus *Allium*.

Mutation breeding is an important tool in crop

improvement of vegetatively propagated crops, particularly in plants with reproductive sterility where this is the only alternative (Broertjes and Harten, 1988). The practical use of this method is reflected in the number of mutant cultivars evolved and put into cultivation. The mutant variety database of FAO/IAEA lists 102 varieties developed through mutation breeding in vegetatively propagated crops in the world which exclude those bred by private nursery enterprise.

In mutation breeding, determining the sensitivity of mutagen is the first step. This is generally determined by finding out LD50 values of the mutagen level. Then the material is treated with the optimum dose (Broertjes and Harten, 1988). Physical mutagens have an advantage over chemical mutagens in that they don't create waste and don't require washing or cleaning the item to remove

the mutagen after use (Khan *et al.*, 2000) especially for asexual plants (Predieri and Divrgilio 2007). Mutation methodology has been used to produce many cultivars with improved economic value and to study the genetics and plant developmental phenomena (Aruna and Adamu, 2010). Mutagens may cause genetic changes in an organism, break the linkages and produce many new promising traits for the improvement of crop plants.

Irradiation has also been successfully used for mutation breeding in various crops and ornamental plants (Song and Kang, 2003) and has proven an adept means of encouraging the expression of recessive genes and producing new genetic variations (Schum, 2003; Song and Kang, 2003; Yoon *et al.*, 1990). Induced mutations serve as a complementary approach in genetic improvement of crops. For improvement of crop by mutation breeding, it is very important to determine a suitable mutagen dose. Seedling growth and cytological characteristics are generally evaluated for testing mutagen sensitivity in plants (Amjad and Anjum, 2002).

Materials and Methods

Experimental Details

- 1. Experimental Site:** The present investigation on Gamma ray induced mutation in Garlic was be carried out during rabi season of the years 2021-2022 at the Horticultural Research Farm, Mondouri, Department of Vegetable Science, Bidhan Chandra Krishi Vishwavidyalaya, Mohonpur, Nadia, average altitude of 9.75 meter above the mean sea level. The site was on a high land with sufficient irrigation and drainage facilities.
- 2. Experimental material:** The parent material to be used in this study is a local cultivar of garlic – *Balagarh local*.

Determination of LD50

Uniform size bulbs of one popular garlic variety namely *Balagarh local* harvested during rabi 2022 irradiated with gamma rays at 0, 5, 10, 15 and 20, 25Gy at the gamma chamber (GC-5000) of the Regional Nuclear Agricultural Research Centre, BCKV, Mohanpur, Nadia, West Bengal, India. The experiment with lethal dose was arranged on a completely randomized block design with six levels (0, 5, 10, 15, 20, and 25 Gy) of gamma radiation with three replications. Observation on different seedling parameters viz., germination percentage, shoot length, root length, fresh weight and dry weight of seedlings were recorded fifteen days after sowing distance with plot size 10 × 15 cm along with untreated control. While exposing to radiation treatment,

bulbs were packed in paper bag and then covered with aluminum foil.

Statistical analysis

The experiment with lethal dose was organized on a completely randomized block design with six levels of gamma radiation (including control) and three replications. The levels of gamma radiation were assigned randomly in the blocks. The one-way ANOVA of all the parameters included in the study was done at 1% significance level to test if the observed averages of treatment levels are significantly different. Probit Analysis was carried out to determine the lethal dose 50 (LD50) of gamma irradiation in Garlic.

Result and Discussion

Effect of radiation on germination and survival

For this cultivar, the sprouting percentage varied significantly ($p < 0.01$) depending on the gamma-radiation dosage. In control (without radiation), germination was initiated earliest and completed within 7 DAP with 99 percent average germination. Among all treatments, in 5Gy, and 10 Gy germination began on the 8th or 9th day after showing and was nearly finished in 15th DAP and 87 and 66 percent germination observed in 5 Gy and 10Gy treatment dosages, respectively. But final matured plants were nil at 10 gy, 15 gy, 20 gy, 25 gy may be due to inactivation of auxin and a decrease in auxin content with increased irradiation dose. According to Khatri *et al.*, 2005 garlic had grown differently on different exposure to radiation. The percentage of living garlic plants decreased and plant growth was more stressed when a higher dose was given. Plants exposed to high gamma-ray radiation doses would lose any mutant or sterilized material. On the other hand, minimal levels of gamma-ray irradiation were able to keep plants alive.

- Plant height:** The maximum dosage of 25 Gy

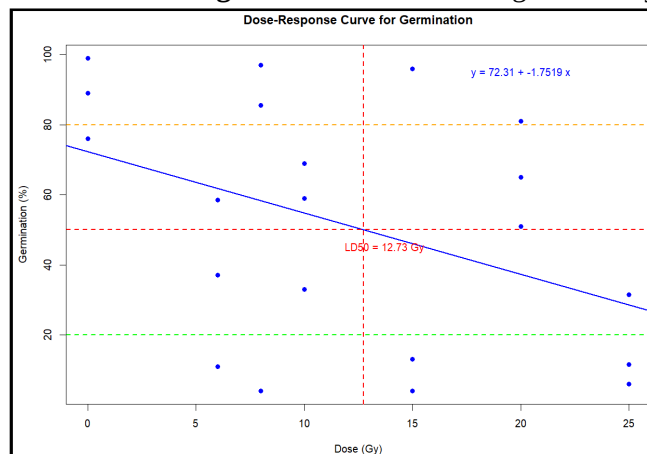


Fig. 1: Dose response curve of germination percentage (Probit analysis).

produced the lowest average plant height (2.33 cm), while the control treatment (0 Gy) produced the highest average plant height (28.667 cm which demonstrated that plant height would decrease with increasing irradiation exposure, That means cell cycles being interrupted at the G2/M phase during somatic cell divisions and/or different types of damage throughout the entire genome could be the cause of the growth reduction caused on by huge gamma-radiation doses (Preussa and Britta 2003). However, it seems that the low

dosage (5 Gy) allowed to continue producing shoots for a longer period of time. (Fig. 3)

- **Effect of gamma radiation on plant growth and bulb traits:** Using biometric measurements of plant parameters such as leaf length and number per plant, the impact of γ ray dosages on phenotypic changes in garlic plants were examined. The main observable morphological characteristics of garlic, such as plant height, leaf length, leaf colour, bulb colour, and bulb shape, as well as other minor characteristics, showed

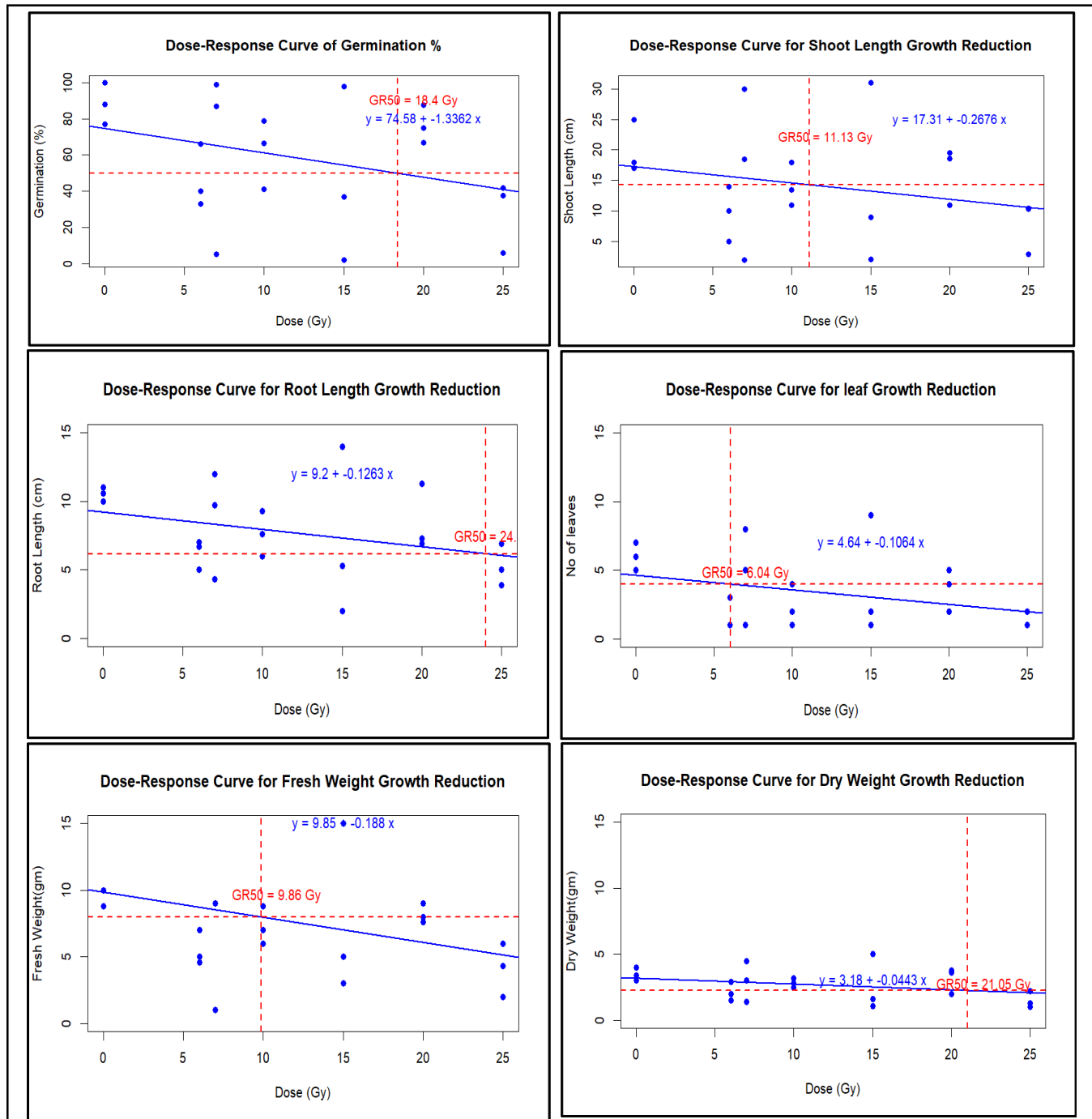


Fig. 2: Dose response curve of different seedling parameters (Linear regression).

an inhibitory effect as radiation doses increased. Same results were reported in cowpea (Singh and Arya 2016) and in marigold (Wang *et al.*, 2007).

- **Number of leaves:** Number of leaves 10 WAP is obtained at the treatment of 5 Gy radiation dose *i.e.* 5.33 and lowest obtained at 25 Gy treatment *i.e.* 1.33.

Radiosensitivity and Lethal Dose

It was suggested that increased radiosensitivity would lead to a higher percentage of mutants forming because more than 50% of mortality might be accomplished with a high dosage of radiation. Since there were notable variations between the observed means of seedlings, the estimation of optimal dosage was valid for different dosage of gamma radiations. Balagarh local LD50 values at a dosage of 12.73 Gy (F-statistic: 4.044 on 1 and 19 DF, p-value: 0.05874) was determined by calculating the living percentage of the treated seeds (Fig. 1) while the GR50 values for germination percentage was arrived at 18.4 Gy using linear regression line. The above-mentioned dose-response curves for every growth parameter showed a negative slope that made it evident how the plants' growth declined as their dose increased. It was found that higher doses of gamma radiation were detrimental to plant vigor and germination rates. Almost all of the measures used for assessing the plants' development showed a similar downward trend as dosages increased. Among the irradiated population, the minimum seedling mortality was noticed with 5 Gy treatment (48 %) which was suddenly increased from 10 Gy to 15 Gy (74%-93%).

The shoot length one-way ANOVA result revealed that, at the 1% significance level ($F=1.464$, p-value= p-value: 0.2411), the observed shoot length averages of different treatment levels were significantly different, and

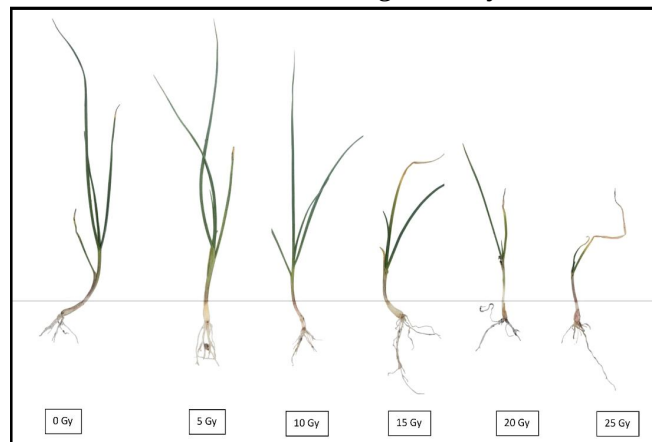


Fig. 3: Effect of varying doses of gamma radiation on different seedling traits of Balagarh Local.

the shoot length GR50 values were determined to be 11.13 Gy. Similar to this, the one-way ANOVA results for the following variables indicated that the observed averages of the various treatment levels differed at the 1 percent significance level: $F=2.477$, p-value=0.132 for the root length; $F=2.558$, p-value=0.01262 for the number of leaves; $F=2.892$, p-value=0.1053 for the fresh weight of the seedling; and $F=2.144$, p-value=0.1595 for the dry weight of the seedling. The GR50 values for root length were determined to be 24 Gy, for leaf count to be 6.04 Gy, for fresh weight to be 9.86 Gy, and for dry weight to be 21.05 Gy. (Fig. 2).

Discussion

The photon particles produced by gamma rays that have the energy to penetrate a molecule and release an electron so that an ionic pair formed (ionization) are what happen when a clove of garlic is exposed to gamma radiation. The physical properties and functions of the cell molecules are altered by this ionization process. According to (Gultom *et al.*, 2020) plant breeders have generally paid little attention to garlic for two basic reasons. First, it is difficult to improve through traditional breeding due to its vegetative mode of reproduction. The second reason is that it takes a long time to go from one generation to another. According to Ahloowalia *et al.*, (2004) 64% of radiation-induced mutant plant varieties are the result of the application of gamma-radiation. On the other hand, different radiation dosages were needed for the generation of mutations and sprout growth in garlic. Thus, the best technological approach and specific gamma-radiation dosages should be determined in order to effectively induce somatic mutagenesis in certain plants or genotypes. Balagarh local LD50 values at a dosage of 12.73 Gy which is satisfied with Pangestuti *et al.*, (2019) who found LD50 value Tawangmangu Baru genotype obtained at dose 7.5 Gy, and LD50 Lumbu Kuning at dose 10 Gy and Broertjes and Van Hanten (1988) found optimum gamma radiation dose of 5 gray for Bhima Omkar and in between 5 to 10 grays for Bhima Purple is identified as LD-50 respectively. Another method for achieving the desired mutations is GR50, which shows a fifty percent reduction in the parameters being studied. In the current study, GR50 values for several seedling characteristics varied from 6.04 Gy to 24 Gy. GR50 does not induce plant mortality, hence it could be utilized efficiently to develop desired mutant plants.

Conclusion

Gamma radiation plays a significant role in inducing mutations in garlic, otherwise difficult to improve through traditional breeding due to its vegetative reproduction and

long generation cycle. The ionization caused by photon particles alters the structure and function of cellular molecules, creating opportunities for somatic mutagenesis. Previous studies have demonstrated that effective mutation induction depends strongly on the applied dosage, with LD50 and GR50 values varying across different garlic genotypes. In this study, GR50 values ranged from 6.04 Gy to 24 Gy, highlighting its potential as a reliable approach to induce desirable variations without causing plant mortality. Thus, determining the most suitable radiation dose for specific genotypes is essential for developing improved mutant lines in garlic and advancing crop improvement strategies

Acknowledgement

I would like to express my sincere gratitude to the Department of Vegetable Science, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, and Regional Nuclear Agricultural Research Centre, BCKV, Mohanpur, Nadia, West Bengal, India, and Division of Agricultural Bioinformatics, The Graduate School, Indian Agricultural Research Institute, New Delhi-110012 India for their invaluable support in providing the necessary tools, materials, resources, office space, and research facilities that made this work possible. Their generous assistance and encouragement have been instrumental in the successful completion of my research.

Reference

- Ahloowalia, B.S., Maluszynski M. and Nichterlein K. (2004). Global impact of mutation-derived varieties. *Euphytica*, **135**, 187-204.
- Amjad, M. and Anjum M.A. (2002). Effect of gamma radiation on onion seed viability, germination potential seedling growth and morphology. *Pak. J. Biol. Sci.*, **39**, 202-206.
- Aruna, H. and Adamu A.K. (2010). The effects of diethylsulphate on some quantitative traits of tomato (*Lycopersicon esculentum* Mill). *Sci. World J.*, **2**, 1-14.
- Benke, A.P., Dukare S., Jayaswall K., Yadav V.K. and Singh M. (2019). Determination of proper gamma radiation dose for creating variation in Indian garlic varieties. *Indian J. Tradit. Knowl.*, **18(3)**, 547-552.
- Broertjes, C. and Van Harten A.M. (1988). Applied mutation breeding for vegetatively propagated crops. *Developments in crop science*. The Netherlands: Elsevier. **12**, 197-204.
- Gultom, T., Simbolon D.L. and Nainggolan W.S. (2020). Effect of gamma rays on phenotypic of garlic cultivar doulu. In *IOP Conference Series: Mater. Sci. Eng. A.*, **725(1)**, 12081.
- Khan, M.A., Ungar I.A. and Showalter A.M. (2000). Effects of salinity on growth, water relations and ion accumulation of the subtropical perennial halophyte, *Atriplex griffithii* var. *stocksii*. *Ann. Bot.*, **85(2)**, 225-232.
- Khatri, A.B.D.U.L.L.A.H., Khan I.A., Siddiqui M.A., Raza S.A.B.O.O.H.I. and Nizamani G.S. (2005). Evaluation of high yielding mutants of Brassica juncea cv. S-9 developed through gamma rays and EMS. *Pak. J. Biol. Sci.*, **37(2)**, 279.
- Kumar, H., Ghawade S.M. and Shivaputra M. (2018). Effect of gamma radiations on growth, yield and quality traits of dolichos bean (*Lablab purpureus* L.). *Int. J. Curr. Microbiol. Appl Sci.*, **6**, 2319-7692.
- Onyeagba, R.A., Ugbogu O.C., Okeke C.U. and Iroakasi O. (2004). Studies on the antimicrobial effects of garlic (*Allium sativum* Linn), ginger (*Zingiber officinale* Roscoe) and lime (*Citrus aurantifolia* Linn). *Afr. J. Biotechnol.*, **3(10)**, 552-554.
- Pangestuti, P.W., Sudarsono S. and Dinarti D. (2020). Determine the effect of gamma irradiation towards the growth of two local garlic genotypes. In *IOP conference series: earth and environmental science*, **497(1)**, 12014.
- Predieri, S. and Divrgilio N. (2007). *In-vitro* mutagenesis and mutant multiplication, Chapter 30. In: Jain SM, Haggman H (eds) *Protocols for micropropagation of woody trees and fruits*. Springer, 323-333.
- Preuss, S.B. and Britt A.B. (2003). A DNA-damage-induced cell cycle checkpoint in Arabidopsis. *Genetics*, **164(1)**, 323-334.
- Schum, A. (2003, August). Mutation breeding in ornamentals: An efficient breeding method. In *XXI International Eucarpia Symposium on Classical versus Molecular Breeding of Ornamentals-Part I*, **612**, 47-60.
- Singh, R. and Arya R. (2016). GNE myopathy and cell apoptosis: a comparative mutation analysis. *Mol. Neurobiol.*, **53(5)**, 3088-3101.
- Song, H.S. and Kang S.Y. (2003). Application of natural variation and induced mutation in breeding and functional genomics: Papers for International Symposium; Current Status and Future of Plant Mutation Breeding. *Korean J. Breed. Sci.*, **35(1)**, 24-34.
- Wang, J., Gonzalez K.D., Scaringe W.A., Tsai K., Liu N., Gu D. and Sommer S.S. (2007). Evidence for mutation showers. *Proceedings of the National Academy of Sciences*, **104(20)**, 8403-8408.
- Yoon, K.E., Im B.G. and Park Y.H. (1990). Effect of gamma radiation on seed germination and androgenesis in *Nicotiana tabacum* L. *Korean J. Breed. Sci.*, **21(2)**, 256-262.