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Naheed Akhtar

Department of Horticulture, PMAS-Arid Agriculture University Rawalpindi,
Pakistan.; e.mail: naheedmalik89@gmail.com

EFFECT OF PHYSICAL AND CHEMICAL MUTAGENS ON MORPHOLOGICAL
BEHAVIOR OF TOMATO (*SOLANUM LYCOPERSICUM*) CV. "RIO GRANDE"
UNDER HEAT STRESS CONDITIONS.

ABSTRACT

Given the importance to tomato production under heat stress conditions in hot climates of Pakistan, the objective of this research work was to study the influence of temperature and application of physical and chemical mutagens on tomato (*Lycopersicon esculentum* L.) CV. "Rio grande" Seeds were treated with chemical mutagen (Ethyl Methane Sulphonate) and physical mutagen (Gamma radiation) . Plants were grown in open field conditions under day/night temperatures regimes at the experimental area of Department of Horticulture, faculty of crop and food sciences, PMAS-AAUR. The experiment was set twenty treatments and four replications in Complete Randomize Block Design. It is observed that by increasing dose of mutagens shows lethal effect and morphological parameters reduced significantly. But lower doses of EMS and Gamma radiation improve thermotolerance capacity significantly. Plants were investigated for different morphological parameters i.e Plant height, no. of leaves, leaf area, relative water contents of leaves, chlorophyll contents, cell viability (TCC Assay) and pollen germination. Among gamma rays 5Kr were the only dose which survived and bear fruits. Among EMS 4mM, 8 mM, 16mM performed better under heat stress conditions than other treatments.

Key words: EMS, gamma rays, heat stress, mutagens, tomato

INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) is one of the most economically important and widely grown plants in Solanaceae family. (Mourvaki *et al.*, 2005).It is grown throughout the world, either outdoors or indoors, because of its wide adaptability and versatility. The estimated world production of tomato is about 161793834 tones.

(FAOSTAT, Database 2012) and total yield is 33681.2 kg/ha. The leading producers are China (with 25.3% of the total production), USA, India, Turkey and Egypt. (FAOSTAT, 2012). The popularity of tomato as fresh and processed food has made it an important source of vitamin A and C in diets. Tomatoes are being consumed throughout the world on a large scale and serve many benefits for heart and other organs. They contain the carotene lycopene, one of the most powerful natural antioxidants (Mourvaki *et al.*, 2005). Its fruits are used in salads or cooked as a vegetable, processed into tomato paste, sauce and puree. In Pakistan, tomato is cultivated in all the provinces but its production is mainly concentrated in Sindh and Balochistan. Total tomato production in year 2012 was 560,000 tones while total yield recorded was 101818.9 (kg/ha) and area harvested 4803680 hectares. (FAOSTAT, 2012). The average yield of tomato in Pakistan is very low as compared to other countries of the world for which many factors are responsible, some of them are failure to ripen, uneven or blotchy ripening, Tomato yellow shoulder disorder but the major problem in different regions of Pakistan is the failure to set fruit or poor fruit set due to day temperatures above 45°C-50°C combined with low humidity and/or drought night temperatures above 21°C or below 10°C. Hot drying winds can add to the problem. Dry soil can cause blossoms to dry up and drop. Cold soils at planting time can stunt growth and delay or eliminate flowering. Lack of air circulation can inhibit the movement of pollen to the flower pistils. The tomato flower happens in the three patterns that are organizational and flowers that are simple appear as well as branched and simple cymes. Flowers number that appears in inflorescence is based on environmental elements including temperature (David *et al.*, 1996). Heat stress is identified as the enhancement in temperature below a threshold level for some time is enough to prompt irreversible harm to crop growth and improvement. As a whole, a temporary increase in temperature 10°–15°C above normal, can lead to heat stress or shock (Wahid *et al.*, 2007). Shortage of tomatoes occurs during summer season due to high temperatures, thus resulting in poor growth, reproductive development and yield (Hall, 1992; Hussain *et al.*, 2006 Singh *et al.*, 2007). When temperature is up, injury of cellules and death may happen within minutes that could be related to a disturbance of cellular structure (Schoffl *et al.*, 1999). When temperature is optimum, damages or death may happen after long term exposure. Direct damages can be happened in high temperatures such as denaturation and aggregation of protein, and enhanced membrane lipids liquidity. Indirect or slower heat damages can be occurred in terms of enzymes inactivation in chloroplast and mitochondria, limitation in protein production, degradation of protein and loss of integrity of membrane (Howarth, 2005). In general, it is obvious that high temperature influences markedly plants anatomy at the tissue, cellular, and sub-cellular levels. The additional impacts of all these alterations in high temperature stress can lead to crop low growth and yield (Wahid *et al.*, 2007). Plant responses to stress and adaptive mechanism form the basis for developing tools/ protocols to improve stress tolerance. Based on the temperature stress response several techniques have been developed to assess the stress effects. Mutation breeding is one of the major technique to develop stress resistant plants, its advantages is that mutants

with multiple traits can be identified. The chances of survival of mutant varieties are much higher under rapid fluctuating climatic conditions. The use of nuclear techniques for developing new varieties under the changing climatic conditions would be the most ideal approach before any new cost effective techniques are developed which are freely available without too many regulations. The purpose of induced mutations is to enhance the mutation frequency rate in order to select appropriate variants for plant breeding. The mutation frequency rate of spontaneous mutations is rather very low and difficult to exploit by the plant breeders. Mutations are induced by physical (e.g. gamma radiation) and chemical (e.g. ethylmethane sulfonate) mutagen treatment of both seed and vegetatively propagated crops. The mutagen treatment breaks the nuclear DNA and during the process of DNA repairs mechanism; new mutations are induced randomly which are heritable. The changes can occur in cytoplasmic organelles and results in chromosomal or genomic mutations and that enable plant breeders to select useful mutants such as flower color, flower shape, disease resistance and early flowering types. A specific advantage of mutation induction is the possibility of obtaining unselected genetic variation, improvement of vegetatively propagated plants when one or few characters of an outstanding cultivar are to be modified.

MATERIALS AND METHODS:

The present study was conducted during March-July 2013 in Horticultural research area of PMAS-Arid Agriculture University Rawalpindi. Seeds of tomato variety Riogrande was treated with physical (Gamma irradiation) and chemical mutagen (EMS). Nine different doses of radiation and EMS were used each dose were considered as a single treatment. Details of doses are shown in Table 1.

Treatments with EMS and Gamma Radiation are as follows Table 1

Treatment	Conc. of EMS	Gamma radiation Conc.
T1	Control (0)	Control(0)
T2	4 mM	5 Kr
T3	8 mM	10 Kr
T4	16 mM	15 Kr
T5	24 mM	20 Kr
T6	32 mM	25 Kr
T7	40 mM	30 Kr
T8	48 mM	35 Kr
T9	56 mM	40 Kr
T10	64 mM	45 Kr

Seeds were subjected to EMS (Ethylmethane sulphonate) mutagenesis. Set of 400 seeds per treatment were imbibed in 200 ml distilled water for 24 hours at room temperature. The seeds were then incubated in 100 ml solution of EMS (Sigma-Aldrich, St. Louis) at different concentrations as shown in table 1. with gentle shaking for 12 h. After the EMS treatment, the seeds were washed twice in 200 ml of 3% sodium thiosulfate buffer for 20 min at room temperature, with gentle shaking, followed by three washes in 200 ml distilled water. The used EMS and wash solutions and all equipment were treated in sodium thiosulfate. Another set of 400 seeds per treatment undergoes the treatment with gamma irradiation in the laboratory of NIFA (Nuclear Institute of Food and Agriculture, Peshawar). The seeds of both treatments were sown in germination trays in Soil: FYM: Sand in the ratio of 2:1:1 and covered with coconut husk. Nursery was ready after one month and transplanted into open field conditions at P x P distance 2.6 feet, all application of required irrigation and management practices were done to evaluate crop for its vegetative and reproductive responses in heat stress conditions. Among them plant height and internodal distance were measured by using measuring tape, no. of leaves and branches were counted manually, Leaf area was measured with the help of digital leaf area meter ADC model AM 100 meter (Analytical Development Company Ltd., Herts, U.K.) at full maturity stage. Leaf chlorophyll concentration will be recorded by Spad-502 chlorophyll meter (Minolta Camera Co. Ltd., Osaka, Japan. Pollen germination was observed by using method of by Abdul-Baki, (1992) by growing pollen in a growth medium containing 0.29 M sucrose, 1.27 mM $\text{Ca}(\text{NO}_3)_2$, 0.16 mM H_3BO_3 , and 1 mM KNO_3 (pH 5.2) to which 0.001% fluorescein diacetate (FDA) was added. Pollen viability can be evaluated within 30 min by determining percent fluorescing pollen in a sample. The RWC of leaves was determined in the fully expanded leaf stage by using the method of (Pirzad *et al.*, 2011). Cell viability test was performed by using TTC analysis, leaf punches were made from the top third leaf at the full leaf expanded stage. After exposing the leaf punches to induction temperature of 38 °C 2 h followed by high temperature of 45 °C for 2 h, they were incubated in 0.2% TTC solution for 4 h and cell viability was measured by taking the absorbance at 485 nm. (Senthil-kumar *et al.*, 2002c; 2002d).

There are no reports in this regard according to our knowledge specifically in Pakistan so the study aims to:

To screen best doses of EMS and Gamma radiation that will be helpful to make stress tolerant tomato mutant plants for future.

Efficiency evaluation of various mutagenesis techniques.

RESULTS AND DISCUSSION

All treatments of Gamma radiation with doses higher than 10 Kr proved to be lethal and complete mortality was observed in seedlings in the 3rd week after sowing. While treatments with Chemical mutagen EMS doses higher than 32 mM were also proved to be lethal. All remaining treatments with control were transplanted into open field condition and evaluate for various parameters and daily mean temperatures were recorded that exceeds up to 45°C and following results were obtained. Results are presented in Table 2

Table 2

Results of morphological traits in response of mutagenic treatments in heat stress conditions

Treatments	Plant height [cm]	Number of branches	Number of leaves	Leaf area [cm ²]	Chlorophyll content in leaves	Inter-nodal length [cm]	RWC	Pollen germination (%)	TCC Analysis
Control	56d	8.25cd	37e	95.94g	62.25a	5.75bc	0.25f	12f	42.6f
GI 5Kr	59.75ab	7.75d	48d	133.4d	60.38ab	6.65a	0.42cd	68b	72.2b
GI 10Kr	28e	4.25e	18fg	103.7f	59.80ab	3.63e	0.35e	0g	34.4g
EMS 4 mM	60.75a	9.5bc	58c	158.5a	51.13c	5.56bcd	0.48cd	75a	75.5a
EMS 8 mM	58.25bc	9.75b	70a	149.7b	57.13b	5.62bcd	0.49cd	68b	65.7c
EMS 16 mM	59.25ab	12a	61b	144.0c	58.73ab	6.08ab	0.59a	66c	66.8c
EMS 24 mM	57.25bc	4e	29f	126.7e	50.88c	4.88cd	0.6a	52d	59.3d
EMS 32 mM	56.25d	2f	20g	132.8d	44.20d	4.75d	0.53bc	34e	45.7e

Plant height (cm)

Through data analysis different doses of gamma radiations and EMS plants were found to have high variability among them. A look on the data revealed that among treatments there was maximum average plant height (60.75 cm) in (EMS 4 mM) whereas minimum average plant height (28 cm) was found in gamma radiation (10 Kr). While EMS doses 8 mM (58.25 cm) and 24 mM (57.25 cm) shows non-significant results with each other. Chromosomes were damaged through radiations and the connection of damaged chromosomes and non-damaged chromosomes have an important correlation with the growth decline (Sparrow and Gunckel, 1955). Considerable phenotypic variations have been shown in studies of induced mutations. The mutation frequency, however, might be controlled by several factors, for instance the mutagen mechanism of action (Griffiths *et al.*, 1993), the size of target gene and composition of nucleotide (Bichara *et al.*, 1995), position of gene in genome (Swoboda *et al.*, 1993),

structure of chromatin and DNA repair efficiency. Higher doses level of mutagens shows lower plant height because these mutagens restricted the somatic cells division and was also observed reduced viability, growth abnormalities and decreased fertility. Thus, there is one most effective dose for each mutagen that brings about the maximum degree of mutagenesis and it depends upon the plant genotype, duration and dose level.

Number of Branches

Data analysis showed both mutagenic treatments had significant differences for number of branches. Minimum number of branches (4 & 2) was recorded in EMS 24mM and 32mM respectively and gamma radiation 10Kr was non-significant with them (4) The number of branches was found to be higher in EMS 16Mm (12) that is highly variable among the other doses and control. With the increase in doses of gamma radiation and EMS a gradually decreasing pattern was observed in case of average number of branches. These results revealed that higher doses level of gamma rays give less secondary branches appeared on plants.

Number of leaves

The interaction of gamma radiations and EMS in case of number of leaves was observed significantly different among all treatments with control. The highest numbers of leaves were counted in EMS 8 mM (70) and 16 mM (61). As the dose level of EMS increased growth parameters decreased and lowest leaves number calculated 29 and 20 in 24 mM and 32 mM respectively. On the other hand gamma radiation 10kr also shows lower no. of leaves 18. Irfaq and Khalid (2001) found that higher doses of gamma radiations had adverse effects on different plant growth characters. A decreasing trend was observed in treatment by increasing doses of gamma radiations and EMS. Table 2 shows clearly that 20 were minimum number of leaves in EMS 32 mM and 18 in 10 Kr Gamma irradiation caused different types of changes in plant structure and function. It depends upon the dose rate and time period of irradiation (Piri *et al.*, 2011). Higher doses might have stopped the enzymes that are necessary for leaves initiation. Another reason found that higher doses blocked cell division by decreasing the rate of physiological processes and genetic material was also damaged (Neary *et al.*, 1957).

Leaf Area

Interaction of gamma radiation and EMS doses gave significant results relating to leaf area. Control had a minimum area of leaves (94.95 cm²) while EMS had maximum (158.5 cm²) leaf area at the dose level of 4 mM. Generally, the leaf area was reduced by increasing the doses of gamma radiations and EMS.

Among the different levels of gamma radiations there was a lowest value of leaf area (133.4 cm²) following by (103.7cm²) at 5Kr and 10kr respectively. Shahin *et al.*, (2011) reported that a dose range of 5-10 gray produced highest leaf area in grapes cultures and at 30 Gy gave lowest leaf area. Extension of cell wall is decreased by stress and high pH which results in the form of structural change in the plant cell wall. Rate of growth for these stressed plants was very low than unstressed plants. Various factors such as biochemical factors were involved in the expansion of leaves and cells. In addition to this, there were many other evidences which supported the slow growth rate of plants according to stress like protein synthesis, processes within the cell wall, cell division and biosynthesis of membranes (Bursens *et al.*, 2000). The results of leaf area were in agreement with the results of Pakorn *et al.* (2009) who demonstrated that leaf area in *Anubias congensis* decreased with the increasing intensity of irradiation due to destruction of genetic material and reduction of cell division and ultimately growth retarded.

Chlorophyll Contents of Leaves

Treated plants showed better growth than the control plants for 5 Kr, 10 Kr and EMS 8 mM and 16 mM as the photosynthetic pigments, that is, Chlorophyll contents were increased by 60.37, 59.8, and 57.1, 58.7 respectively, over the control plants (39.1)., with increasing dose of EMS treatment, the leaf Chlorophyll contents decreased continuously and that decrease was recorded up to 44.2 for 32 mM EMS. These results were similar in findings with Jitendra *et al.*, 2012 by using gamma radiation and EMS in Safflower shows higher number of chlorophyll a and b contents as the intensity of mutagens decreases. Moreover Exposure to high temperature induced chlorophyll loss, led to marked decline in the photochemical efficiency of PSII and caused considerable degradation of Rubisco in plant leaves. Plants with lower doses of mutagens shows protective response to heat stress, which involves structural alterations in the photosynthetic apparatus as compared to higher doses. Prolonged irradiations damage to reaction centers is usually followed by chlorophyll loss (Krause, 1998). Exposure of leaves to higher temperatures causes inhibition of both photosynthesis and respiration hence during the vegetative stage chlorophyll content degradation ultimately lowers carbohydrate synthesis. The heat stress directly affects the heat sensitive sites, mainly Oxygen Evolving Complex of Photosystem II and Rubisco activase. It leads to subsequent indirect effects, such as changes of the redox status of individual components on thylakoid membrane in chloroplast and increase in production of Reactive Oxygen Species (ROS). Hence, the redox signaling plays the crucial role in enhancement of alternative electron pathways such as cyclic electron flow as well as triggering the signal transduction pathways resulting to heat stress response. The redox signaling in chloroplast is closely associated with ROS signaling, which interferes with regulation also out of chloroplast. Marian Brestic, (2013)

Inter-nodal length

Data regarding inter-nodal length depicted significant interaction between the gamma radiations and EMS applications. It was found that inter-nodal length was minimum in gamma radiation treatment 10 Kr (3.63) and EMS treatment 32mM (4.75). Among different treatments, inter-nodal length was maximum 6.65 cm in 5 Kr of gamma radiation and 6.08 cm EMS 16 mM. With the increasing level of gamma radiations and EMS there was a decrease in the inter-nodal length and ultimately plant height. The findings of Motilal *et al.* (2012) are matched with the findings of the current study. He explained that there was a decrease in inter node length with the increase dose level of EMS in *asteracantha longifolia* L. Major impact of high temperatures on shoot growth is a severe reduction in the first internode length resulting in premature death of plants (Hall, 1992). For example, sugarcane plants grown under high temperatures exhibited smaller internodes, increased tillering, early senescence, and reduced total biomass (Ebrahim *et al.*, 1998).

Relative Water Contents

By observing results after statistical analysis for relative water contents of leaves reveals that higher RWC was observed in treatments EMS 16 mM and 24 mM 0.6 and 0.59% while non-significant results were obtained in treatments with 4 mM and 8 mM. Among gamma radiation treatments 5 Kr (0.42) shows higher RWC than 10 Kr (0.35) while control (0.25) shows least value for RWC. It shows the plants which are treated with mutagens have more ability to absorb water when transpiration rate were higher in heat stress conditions as compared to plants which are in control. High temperature causes loss of cell water content for which the cell size and ultimately the growth is reduced. Reduction in net assimilation rate (NAR) is also another reason for reduced relative growth rate (RGR) under high temperature but mutagen treatments improves the ability of more intakes of water and nutrients from soil to maintain turgidity and overall plant vigor in stress conditions. As the heat stress hampered the leaf water content, plants exposed to heat stress indicated an accumulation of several heat stable proteins which appeared to be associated with heat tolerance. Results are similar in correspondence with the results of Morales *et al.*, (2003) who stated that preconditioned tomato plants with heat indicated a better osmotic adjustment and maintained leaf water potential than non-preconditioned ones.

Pollen Viability test

Data presented in Table 2 shows that treatment with lower doses of EMS provides more viable pollen growth as compared to control under heat stress conditions. While 5 Kr dose of gamma radiation also increases the number of viable pollen grains. EMS 4 mM (75%) shows higher percentage of viable pollen at temperature above 40C and EMS 8 mM and gamma radiation dose 5 Kr shows

non-significant result (68%) with each other but significantly different from control (12%). In tomato, a dramatic decrease in fruit set in response to heat stress has mainly been attributed to the impairment of pollen development occurring in anthers 8–13 days before anthesis, corresponding to the shift from pollen mother cells to uni-cellular microspores. This heat stress sensitivity of the male reproductive organs has been observed after short episodes of high temperatures at 40 °C and after chronic exposure to 32/28°C (day/night), which is just few degrees above the optimal physiological growth range of 26/22 °C (day/night) observed by Filomena *et al.*, (2013). But mutagen treatment dramatically increases the ability of plants to produce viable pollen at higher temperature stress conditions.

Cell Viability (TCC Assay)

TCC assay is useful technique to assess cell viability at higher temperature stress conditions. By showing results it's confirmed that applications of EMS and Gamma radiation significantly increases the viability of cells under heat stress. Application of EMS 8mM has significantly higher number of viable cells 75.5 while 16 mM and 24 mM show non-significant results with each other. Gamma radiation dose 5 Kr also increases thermostability of cells at higher temperatures as compared to control conditions. The TTC reduction reflects overall cell viability, which is often related to extent of generation of cytotoxic compounds, integrity of macromolecules and membrane-bound organelle integrity (Wu and Wallner, 1983). Further, this technique is amenable for large-scale screening.

CONCLUSION

From all the tests and procedures, our results indicated that significant genetic variation could be created by physical and chemical mutagens. Further, the TTC test, pollen viability test, and chlorophyll contents could be used to assess genetic variability in this mutant population under heat stress conditions.

REFERENCES

- Abdul Baki A., R. John. 1992. Pollen viability and fruit set of tomato genotypes under optimum high temperature regimes. Hort. sci., 30: 115- 117
- Bichara, M., S. Schumacher and R. P. P. Fuchs. 1995. Genetic instability within monotonous runs of CpG sequences in *Escherichia coli*. Genetics, 140: 897-907.
- Britt, A. B., J. J. Chen, D. Wykoff and D. Mitchell. 1993. A UV-sensitive mutant of *Arabidopsis* defective in the repair of pyrimidine-pyriminone (6-4) dimers. Sci., 261: 1571–4.
- Burssens, S., Himanen, K. Cotte, B. Beeckman, T. Vanmontagu, M. Inze, D., Verbuggen, N. 2000. Expression of cell cycle regulatory genes and morphological alterations in response to salt stress in *Arabidopsis thaliana*. Planta, 211: 632-640.
- David FD, Heidmann J, Heinen C, Lamp B, Martin J, Rost T, Silady R, Tong K, Tassel DV. 1996. Section of Plant Biology Division of Biological Sciences, University of California, DAVIS,

- Ebrahim, M.K., Zingsheim, O., El-Shourbagy, M.N., Moore, P.H., Komor, E. 1998 Growth and sugar storage in sugarcane grown at temperatures below and above optimum.-J. Plant Physiol. 153: 593–602,
- Filomena ,G., Mieke W. A., Celestina, M. and Ivo, R. 2013. Ensuring Reproduction at High Temperatures: The Heat Stress Response during Anther and Pollen Development, Plants, 489-506; doi:10.3390/plants2030489
- Griffiths, A. J. F., J. H. Miller and D. T. Suzuki. 1993. An introduction to Genetic Analysis. pp. 43-52.
- Hall, A. E., 1992. Breeding for heat tolerance. Pl. Breed. Rev., 10: 129–68
- Howarth CJ. 2005 Genetic improvements of tolerance to high temperature. In: Ashraf M, Harris PJ, (eds). Abiotic Stresses: Plant Resistance Through Breeding and Molecular Approaches. Howarth Press Inc., New York,
- Hussain, T., I. A. Khan, M. A. Malik and Z. Ali, 2006. Breeding Potential for high temperature tolerance in corn (*Zea mays* L.). Pakistan J. Bot., 38: 1185–95.
- Irfaq, M. and N. Khalid. 2001. Effect of gamma irradiation on some morphological characteristics of three wheat cultivars. J. Biol. Sci., 10: 935-937.
- Jitendra, K. P., Preeti S., Ram S. Y. and Ram G., 2012. Chlorophyll Fluorescence Spectra as an Indicator of X-Ray + EMS-Induced Phytotoxicity in Safflower. Hindawi Publishing Corporation Spectroscopy: An International Journal Volume 27 (2012), Issue 4, Pages 206–213
- Krause, G. H. 1988. Photoinhibition of photosynthesis: an evaluation of damaging and protective mechanisms. *Physiologia Plantarum* 74, 566–74.
- Marian Brestic, In book: Plant signaling: Understanding the molecular cross-talk, Edition: 2013, Chapter: Heat Signaling and Stress responses in photosynthesis, Publisher: Springer Verlag, Editors: Khalid Rehman Hakeem, Reiazul Rehman, Inayatullah Tahir
- Morales, D., Rodríguez, P., Dell'Amico, J., Nicolás, E., Torrecillas, A., Sánchez-Blanco, M. J. 2003 High-temperature preconditioning and thermal shock imposition affects water relations, gas exchange and root hydraulic conductivity in tomato.-*Biol. Plant.* 47: 203–208,
- Motilal B., P. Jogeshwar, Mishra, R. R. and Rath, S. P. 2012. Analysis of EMS induced in vitro mutants of *Asteracantha longifolia* L. Nees using RAPD markers. *Indian j. of Biotechnology* vol., 11, pp 39-47.
- Mourvaki, E., S. Gizzi, R. Rossi and S. Rufini. 2005. Passionflower Frui., A New Source of Lycopene. *J. Med. Food*: 104-106.
- Neary, G. J., H. J. Evans and Tonkinson. 1957. Mitotic delay induced by gamma radiation in Broad Bean root meristems. *Nature*, 179: 917-918.
- Pakorn, T., T. Taychasinpitak, C. Jompuk and P. Jompuk. 2009. Effects of Acute and Chronic Gamma irradiations on *in vitro* culture of *Anubias congensis*. *J. Nat. Sci.*, 43: 449-457.
- Piri, I., B. Mehdi, T. Abolfazl and J. Mehdi. 2011. The use of gamma irradiation in agriculture. *Afr. J. Micro. Biol. Res.*, 32: 5806-5811.
- Pirzad AR, Shakiba MR, Zehtab-Salmasi S, Mohammadi SA, Darvishzadeh R, and Samadi A. 2011. Effect of water stress on leaf relative water content, chlorophyll, proline and soluble carbohydrates in *Matricaria chamomilla* L. *Journal of Medicinal Plants Research* 5(12): 2483-2488
- Schoffl, F., R. Prandl, and A. Reindl. 1999. Molecular responses to heat stress. In: Shinozaki K, Yamaguchi-Shinozaki K, (eds). *Molecular Responses to Cold*, selected on the basis of temperature-induction response. *Theor. Appl. Genet.*, 99:359–36.
- Senthil, K., M. M. B. Raju, and M. Udayakumar M. 2002c. Development of abiotic stress tolerant tomato and capsicum lines: a novel approach using TTC and TIR techniques. Paper presented in 'International conference on vegetables, 11–14 November 2002, Bangalore, India, No. II-180-O, p 51.
- Senthil, K., M. Srikanthbabu, V. P. Aarati and M. Udayakumar. 2002d. Identifying tomato (*Lycopersicon esculentum* L.) population for abiotic stress tolerance: A screening technique using TTC. Paper presented in 'National seminar on emerging trends in horticulture 14–15 February 2002, Annamalainagar, India, No. 3.6, p 86.
- Shaffer, S. D. and L. L. Wallrath and S. C. R. Elgin. 1993. Regulating genes by packaging domains: Bits of heterochromatin in euchromatin. *Trends in Gen.*, 9: 35-40.
- Shahin Abad, L. M., V. Rashidi, and A. R. Eivazi. 2013. Evaluation of drought tolerance indices in corn genotypes (*Zea mays* L.). *International Journal of Current Research* 5:85-88.
- Singh, R. P., P. V. Vara Prasad, K. Sunita, S. N. Giri and K. R. Reddy, 2007. Influence of high temperature and breeding for heat tolerance in cotton. *Adv. Agron.*, 93: 313–85.
- Sparrow, A. H. and Gunckel, J. E. 1955. The effect on plants of chronic exposure to gamma radiation from radiocobalt. *Proc. Intl. Conf. Peaceful Use of Atomic Energy, Geneva*, 12: 52-59.
- Swoboda, P., Hohn B. and Gal S. 1993 Somatic homologous recombination *in planta*: the recombination frequency is dependent on the allelic state of recombining sequences and may be influenced by genomic position effects. *Mol. Gen. Genet.*, 237, 33–40.

- Swoboda, P., B. Hohn and S. Gal. 1993. Somatic homologous recombination in plants: The recombination frequency is dependent on the allelic state of recombining sequences and may be influenced by genomic position effects. *Mol. Gen. Genet.*, 237: 33-40.
- Wahid A, Gelani S, Ashraf M, Foolad MR. Heat tolerance in plants: An overview. *Environ Exp Bot.* 2007;61:199–223.
- Wu, M. T. and Wallner, S. J. 1983 Heat stress responses in cultured plant cells. Development and comparison of viability tests; *Plant Physiol* 72 : 817–820.