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Variation Caused by Gamma Rays and Ethyl Methane Sulfonate on the Morphological Characters, Lycopene and Vitamin C Contents of Tomato

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Abstract

Modern techniques of using mutagens to create modifications in the plants for the development of better surviving traits with high yield had gained popularity rather than conventional breeding techniques. Variability in the morphological traits, fruit set percentage and nutrient profile of tomato fruit was caused by the Ethyl Methane Sulfonate treatments. Water soaked seeds of *Solanum lycopersicum* Mill. were treated with gamma rays 5 Krad, 10 Krad and EMS of concentration 4 mM independently. Morphological parameters were observed before fruit picking and fruits were picked at the breaker stage and analysis and observation were made of fruits for vitamin C and lycopene contents. These results were significant at 5% level. Results revealed that EMS 4 mM concentrations showed better results as compared to Gamma ray treatments. Data was analyzed by statistix 8.1 softwrae.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) belongs to family Solanaceae. It is a perennial crop but worldwide grown as an annual crop for its fruit (Grant and Owens, 2002) and ranks second important crop after potato among vegetables. According to an estimate total world area under tomato cultivation is 4.58 billion hectares producing 150.51 billion tonnes and area under tomato cultivation in Pakistan during the year of 2011-2012 was 52,300 hectares producing 530,000 tonnes. Tomato is also known as poor man's orange because it is highly nutritious and contains 74.97 mcg vitamin A, 2.56 mg vitamin B and 24.66 mg vitamin C (Imran *et al.,* 2012). Tomato is a source of polyphenols, tocopherols, rutin and naringin flavonoids (Fruscinate *et al.,* 2007). Morphological and nutritional value of tomato can be improved by utilizing breeding techniques but this method is time consuming on other hand mutagenesis is the phenomenon providing same benefit. Mutagens have ability to cause variation in the genetic makeup of species and this phenomenon has been exploited to create modifications in tomato but little work is done in regard of changes arise in the nutritive profile because of mutagen application in M1 generation. Mutagenesis provides facility to improve one or more desired traits without changing the rest of genetic makeup (Mahandjiev *et al.,* 2001). Among physical mutagens, Gamma rays are categorized as ionizing radiations of high energy possessing power of penetration in the cells and causing production of free radicals. As this ability of gamma rays is responsible for generating masked effects on morphology, anatomy, biochemistry and physiology of plants depending upon the dose (Jan *et al.,* 2012). Gamma radiations at a dose of 10 Krad improved morphological characters i.e. plant height and productivity (Mahmoud and Tawaty, 2006). Okra seeds of Sabahia or Balady varities treated with 400 Gy improved all morphological characters, pod length, number of pods per plant, seed weight and seed yield (Hegazi and Hamideldin, 2010). But higher doses of 600 and 800 Gy adversely affect the morphological characters of tomato and okra plants (Norfadzrin *et al.,* 2007). Among chemical mutagens alkalyting agents especially Eethyl Methane Sulfonate is widely used and greatly suggested for seed treatment because it causes point mutations and produces wide range of alleles within a relatively small population (Emmanuel and Levy, 2002). Another advantage of EMS is to cause mutation at single locus but without causing harmful mutations of related genes (Alcantara *et al.,* 1995). Mahmoud and Twaty (2006) reported that an increase in carotenoid content by 85.71% when tomato seeds were treated with 4 Krad Gamma rays. EMS having capability to induce point mutations which will be able to affect color and sugar contents of tomato. Wilde *et al. (*2012) reported delayed ripening of tomato obtained by affecting β-galactosidase, expansin genes and ethylene receptors and this result is in accordance with Gady *et al.* (2012) that point mutations gave mutants having less carotenoid synthesis and fruit remained yellow for 3days in post breaker stage.

Selection of adequate doses is very important as higher doses of both physical and chemical mutagens cause delay in germination, reduce root shoot length and loss of fertility (Kodym and Afza, 2003). Present study was done with an objective of analyzing the changes caused in the tomato morphology and vitamin C contents by different doses of Gamma rays and EMS seed treatment.

MATERIALS AND METHODS

Experimental trial was conducted at the vegetable research area of Horticulture department of PMAS-AAUR during the year of 2013. This area lies in semi –arid zone which is characterized by humid to sub-humid climate. Tomato seeds of cultivar "Rio Grande" were treated with 5 KRad and 10 Krad of Gamma rays from Nuclear Institute of Food and Agriculture (with the Co-60 source) and with 4 mM concentration of chemical mutagen "Ethyl Methane Sulfonate" independently then kept for 12 hours at room temperature followed by washing with distilled water. After EMS treatment seeds were sown in the germination trays following four replications for each treatment and kept inside the green house. At 2-4 leaves stage seedlings were shifted to lath house for hardening and kept there for a week and only survived seedlings were transplanted into the field of each treatment comprising four replications. Plants came into bloom after 2 weeks of transplantation and bearing was started after one and a half month of transplantation. Germination percentage was counted right after germination and seedling height measured by using measuring scale right before transplantation in the field. Fruits were picked at breaker stage from each replication and brought to post-harvest laboratory for Lycopene and Vitamin C analysis. Mature plants were analyzed for Plant height (cm), No. of Leaves, Leaf Area, Chlorophyll contents and Fruit Set percentage. Experiment was conducted following Randomized Complete Block Design (RCBD) and Analysis of Variance was applied. Least significant difference at 5% level was used to evaluate difference among treatments (Steel *et al.,* 1997).

RESULTS AND DISCUSSION

As highest germination percentage was recorded in untreated seeds (72%) followed by EMS 4 mM (62.50%), 5 Krad (38.75%) and 10 Krad (10%). It had been observed that failure in germination occurred due to the inability of seeds to develop their roots and this phenomenon had been observed in both EMS treated and Gamma irradiated seeds. By increasing the dose of mutagens reduction in germination was occurred and these results are in accordance with the results of Girija and Dhanavel (2009). Adamu and Aliyu (2007) also observed 50% reduction in germination occurred at dose of 5 Krad and 10 Krad in Gamma irradiated seeds. According to Qurany and Khan (2009) lower percentage of germination resulted due to change in metabolic functions of cells along with negative effects on growth regulators in response to higher dose of mutagens. It is concluded that by increasing mutagen dose reduction in germination of seeds result due to damaging effects of mutagens.

Seedling height was found maximum in untreated (10.75 cm) and EMS 4 mM (10.625 cm) seedlings followed by 5 Krad (8.75 cm) and 10 Krad (4cm). Maximum plant height was observed in plants of EMS 4 mM (60.75 cm) as compared to Gamma 5 Krad (59.75 cm) and minimum height was given by 10 Krad (28 cm) Gamma plants. These results are in accordance with the results of Aliero (2006) who found reduction in plant height at higher doses of hydroxylamine.

According to Girija and Dhanavel (2009) reduction in height of plants results due to failure of auxins synthesizing mechanism and chromosomal abnormalities. According to Nofadazrin *et al.* (2007) higher doses of gamma radiations cause negative impact on morphology of plants propagated through treated seeds which are in support of these results. Vegetative growth increases in response to lower doses of gamma rays because these ionizing radiations cause synthesis of certain amino acids like lysine and phenylalanine and hormones like auxins which hastens multiplication rate of cells. Lower doses of gamma rays enhance certain metabolic functions which help plant to overcome temperature and light fluctuations and their extremes thus facilitating plant to improve its vigor (Jan *et al.,* 2011). Studies have shown that plant responses to mutagens doses are plant specific as well. Maximum number of leaves were produced by EMS 4 mM (58) plants followed by 5 Krad (48.75) mutagen dose and their number is higher than non-treated plant (37.25) and minimum leaves were given by 10 Krad Gamma dose (18.75). Adamu and Aliyu (2007) reported reduction in number of leaves in tomato in response to higher doses of alkalyting mutagens like dimethylsulphate.

Menshah *et al.* (2007) indicated the development of less number of leaves per plant in sesame at higher doses of mutagen application. EMS of concentration 0.7% also caused an increase in number of leaves per plant in carnation (Roychowdhury and Tah, 2011). Plants developed from 5 Krad Gamma irradiated seeds produce less number of leaves (48.75) as compared EMS treatments but showed an improvement in this trait as compared to non-treated plants (37.25). It had also been observed that by increasing the dose of gamma radiations to 10 Krad caused reduction in number of leaves (18.75). Jan *et al.* (2011) also enlisted reduction in number of leaves at 15 and 20 KGy in *Psoralen corylifolia* L. These results are in accordance with the results of Falusi *et al.* (2013) reported an increase in number of leaves per plant when seeds of Capsicum annum were treated with fast neutrons of 1.5 × 104 n cm-2 s-1 for 60 minutes. An increase in number of leaves at lower doses of mutagens is attributed to the enhancement of growth promoters and higher doses of mutagens cause elevation of growth inhibitors which arrest cell cycle at G2 metaphase (Jan *et al.,* 2011). The presented results suggest EMS 8 mM is the best dose to increase number of leaves in tomato as compared to higher doses of EMS and Gamma 5 Krad.

Gamma rays and EMS treatments had shown leaf area within ranges of 103.79 cm^2 -158.56 cm^2. Highest leaf area was found in plants of EMS treatment as compared to non-treated and gamma ray treatments. Jabeen and Mirza (2002) found an increase in leaf area of capsicum when its seeds were treated with 0.01% EMS. Among gamma radiations, 5 Krad (133.4 cm^2) showed better results than 10 Krad (103.79 cm^2) gamma treatment and non-irradiated (95.94 cm^2) as well. According to Jan *et al.* (2012) lower doses of gamma rays promote cell division and growth in plants by stimulation of growth enhancing hormones and activating the antioxdative capacity of cells to cope with environmental stresses. Contrary to this, higher doses arrest somatic cell division and inhibit growth. Gamma radiations interact with cells and atoms and produce free radicals. Theses free radicals either cause modification or destruction of cell organelles eventually become the cause of change in metabolic process of cell. This change also affects physiological and biochemical processes leading to an increase or decrease in cell division and multiplication rate. Jan *et al.* (2011) observed an increase in leaf area of *Psoralen corylifolia* L. at 1000 Krad but found significant reduction in leaf area at higher doses of 1500 and 2000 Krad which are in accordance with the presented results. EMS had been reported to cause changes in leaf area of melon (Tadmor *et al.,* 2008). Qurany and Khan (2009) found maximum increase in leaf area of *Eruca sativa* L. at a concentration of 3 mM sodium azide. Therefore, lower doses of mutagens have ability to increase leaf area but higher doses have inverse effect as well.

Highest chlorophyll contents were observed in non-treated plants (62.25%). Gamma irradiated plants 5 Krad and 10 Krad (60.37, 59.8 respectively) did not show significant difference for chlorophyll contents within treatments and found better than EMS 4 mM (51.125) treatment. Mahmoud and Twaty (2006) reported an increase in chlorophyll contents of tomato by the irradiation of seeds with 2 and 4 Krad. This statement is contradictory to discussed results as irradiated plants did not show an increase in chlorophyll contents and these values were also a bit lower than untreated one (62.25). These results can be supported by Mehandjiev *et al.* (2001) findings who reported low mutation frequency in chlorophyll contents when pea seeds were irradiated by gamma rays.

Qurany and Khan (2009) reported an increase in chlorophyll contents when tomato seeds were treated with 1 mM sodium azide. Higher doses of sodium azide also caused reduction in chlorophyll value. Results of present study are similar to Qurany and Khan (2009) but EMS 16 mM has shown different trend because of the mutation at different alleles in genome. Jabeen and Mirza (2002) found an increase in chlorophyll contents of Capscium annum when treated with 0.1% EMS for three hours but Ethyl Methane Sulfonate above and below 0.1% showed lower values. According to Abdullah *et al.* (2009) by the gamma irradiation of rhizomes of Thai tulip, chimeras are produced because of modification of chlorophyll contents.

EMS 4 mM plants gave 95% fruit set followed by 5 Krad (80%) which were higher than non-treated plants (55%). 10 Krad Gamma treatment did not give production during whole life cycle of plants. Many previous studies showed an increase in fruit set percentage of *Capsicum annum*, black gram, roselle and other different crops when treated with different types of mutagens at lower concentrations (Gandhi and Devi, 2014; Arulbalachandran *et al.,* 2010; Sheriff *et al.,* 2011). According to Mahmoud and Tawaty (2006) 4 krad of Gamma rays seed treatment and sodium azide at a concentration of 1 mM caused an increase in fruit set percentage in tomato when seeds were treated with these two mutagens independently. Increased fruit yield is associated with the genetic variability caused by mutagens due to biochemical and physiological mutations of secondary level which are usually of permanent nature (Dhakshanamoorthy *et al.,* 2010).

Hegazi and Hamideldin (2010) reported enhanced productivity of okra by irradiation of seeds with 400 Gy (40 Krad) before sowing. According to Arulbalachandran *et al.* (2010) radiations modify genetic make-up of plant by deleting or substituting base pairs of DNA which result into either improvement or reduction of quantitative traits like yield and fruit set percentage due to additive gene phenomenon.

Fruits taken from 5 Krad Gamma treatment (2.675 mg) and EMS 4 mM (2.825 mg) showed lower lycopene as compared to non-treated fruits (3.375 mg). Lycopene starts accumulating in tomato fruit at green stages and reaches to its maximum at ripe stage; it is the dominating carotene of tomato along with small amounts of β carotenes. Pytoene synthase (PSY 1) and Phytoene desatures (PDS) facilitate lycopene accumulation and their amount increase in the fruit during fruit maturation (Ronen *et al.,* 1999). EMS 32 mM showing higher concentration of lycopene depicts higher level of PSY 1 and PDS comparatively, due to the mutations at gene level. Studies have shown that lycopene saturation results due to divergence of genes at the time of fruit development and by the increased activity of enzymes that cause blockage in synthesis of carotenoids. Lower values of lycopene are also connected with the high temperature during fruit development as Dumas *et al.* (2003) stated, higher temperatures decrease lycopene synthesis and inhibition is caused at 35 ^{0}C or above. EMS and Gamma treatments showed poor performance in terms of lycopene production under high temperatures as lycopene was severely decreased due to the injury of fruit tissues caused by elevated temperatures during month of June.

Highest ascorbic acid contents 13.68 mg were found in the fruits picked 5 Krad Gamma treatment which was higher than the amount of vitamin C observed in non-treated fruits (9.3930 mg) but fruits picked from EMS 4 mM (5.98 mg) showed minimum Vitamin C contents. Ascorbic acid acts as an antioxidant and performs significant role in cell enlargement and growth (Loannidi *et al.,* 2008). This statement is supported by the presented results as fruits of comparatively larger size (Control, 5 Krad).

According to Stevens *et al.* (2007) and Matteo *et al.* (2010) ascorbic acid is a quantitative trait and controlled by various genes along with this higher concentration of ascorbic acid is related to the disintegration of cell wall pectin which on the other hand leads to the reduction of fruit firmness. This statement is in accordance with the findings of present study as treatments 5 Krad, EMS 8 mM and 16 mM had shown relatively higher Vitamin C and lower firmness. Despite of these results non-treated fruits and fruits taken from treatment of EMS 4 mM concentration showed deviation from this statement.

Synthesis of more reducing sugars also plays an important role in the production of ascorbic acid. Some genes are involved in prevention of oxidation of AA by decreasing the number of free radicals leading to increase AA level in the fruits (Matteo *et al.,* 2010).

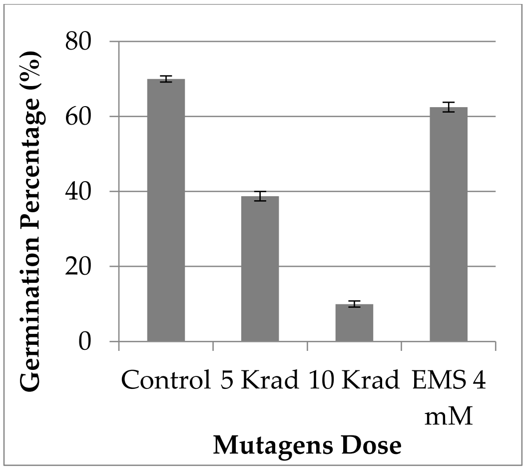

Figure 1: Gamma rays and EMS effects on germination percentage (%).

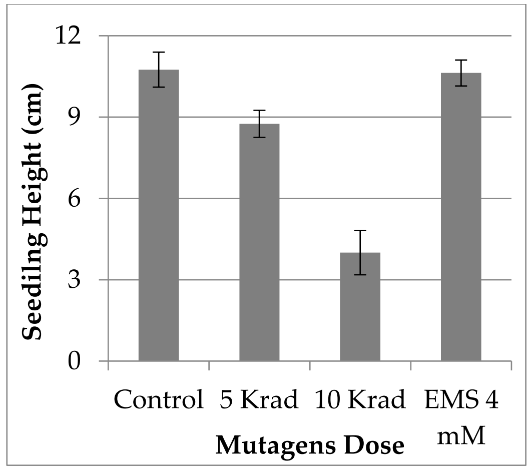

Figure 2: Gamma rays and EMS effects on seedling height (cm).

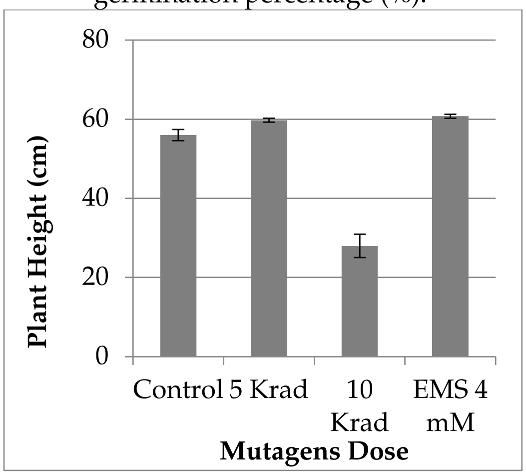

Figure 3: Gamma rays and EMS effects on plant height (cm).

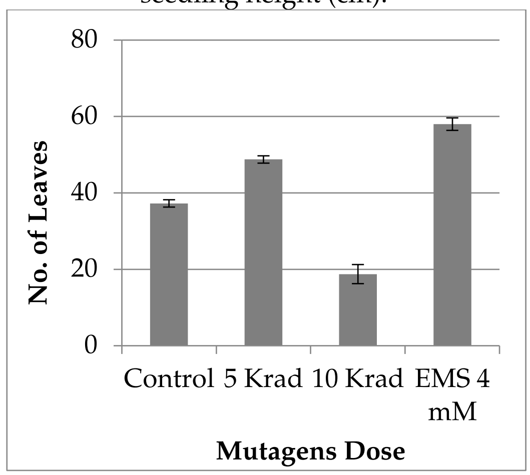

Figure 4: Gamma rays and EMS effects on number of leaves.

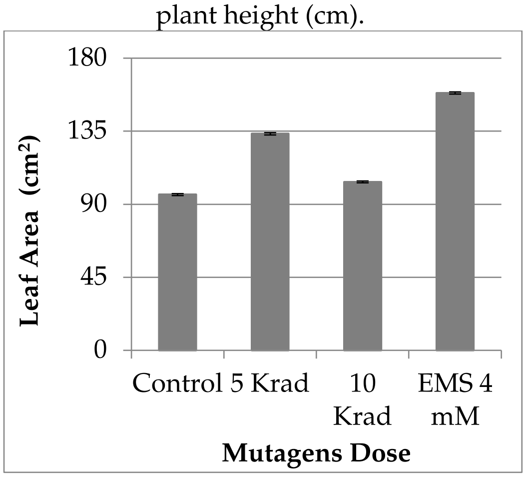

Figure 5: Gamma rays and EMS effects on leaf area (cm^2).

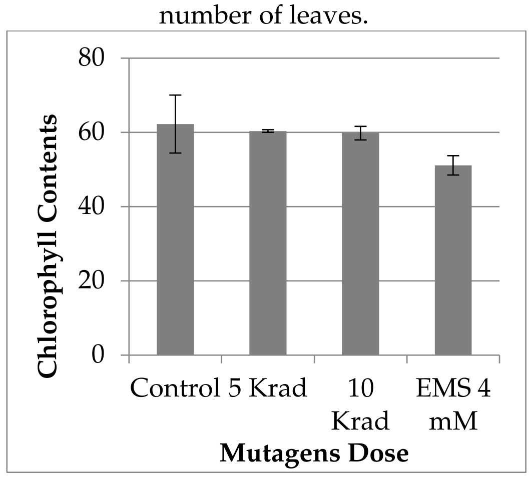

Figure 6: Gamma rays and EMS effects on chlorophyll contents.

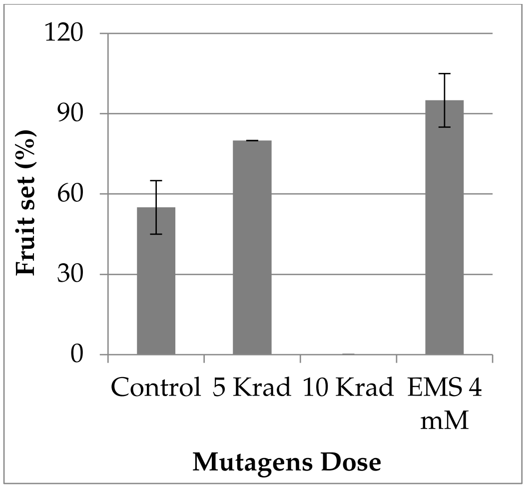

Figure 7: Gamma rays and EMS effects on fruit set (%).

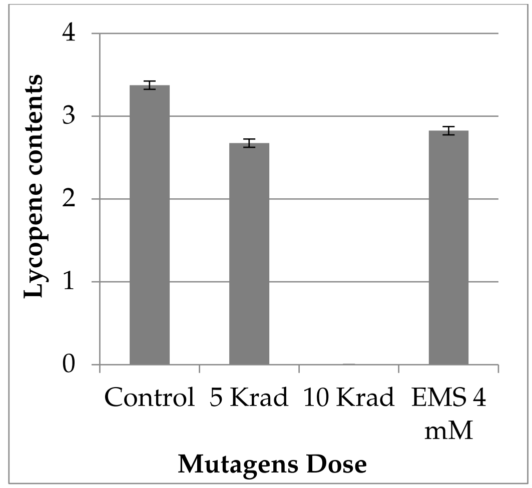

Figure 8: Gamma rays and EMS effects on lycopene contents (mg/100 ml of juice).

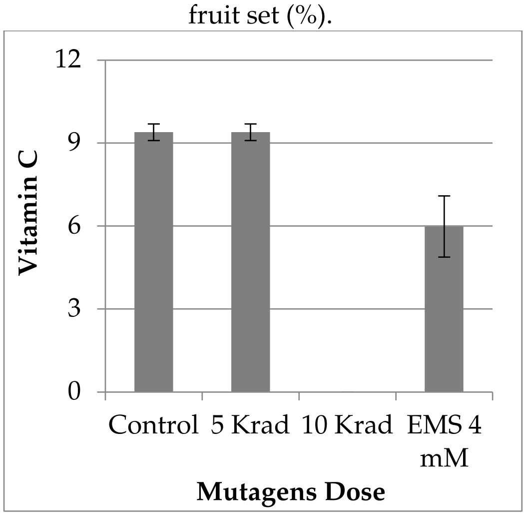

Figure 9: Gamma rays and EMS effects on vitamin C contents (mg/100 ml of juice).

CONCLUSION

Seeds were treated EMS 4 mM concentration performed better in comparison to Gamma ray treatments except for Vitamin C contents and chlorophyll contents. Among gamma ray treatments 5 Krad dose found to be better for all traits comparatively but 10 Krad did not switch to reproductive stage for the whole life cycle.

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