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# Effect of Gamma Rays and EMS on Phytochemical Constituents in Chilli (*Capsicum annuum* (L). Var- $K_1$ on $M_2$ Generation

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**T.ARULDOSS. L.MULLAINATHAN**

*Department of Botany, Annamalai University, Annamalai Nagar-608 002 Tamilnadu, India.*

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**ABSTRACT**

The present study was conducted in order to find out the effect of Gamma rays and EMS on phytochemical constituents in chilli on $M_2$ generation. The phytochemical constituents induced chlorophyll, Capsaicin, Oleoresin, Capsanthin and Ascorbic acid. The seeds were treated with different concentration of (30kR, 40kR and 50kR) Gamma rays and (20mM, 30mM and 40mM) of EMS. The $M_1$ seeds were used to rise $M_2$ generation. The results of the present study suggest that concentration of Gamma rays treatment at (40kR) and (30mM) EMS treated plants increased the chlorophyll, Capsaicin, Oleoresin, Capsanthin and Ascorbic acid contents in chilli.

## INTRODCTION

The genus capsicum, which originates from tropical and humid zones of central and southern America, belongs to the solanceae family and possesses high economic value. Capsicum is one of the oldest and most popular vegetables and spices in the world. Commonly known as Chilliles, provide widest range of physiological effects however results of some studies conducted to explore the beneficial effects of chillies were positive and some were negative. Though there are several common uses and benefits, the most popular application is as a spice (Pawar et al., 2011). Induced mutagenesis has been recognized as the most efficient method for induction of morphological and genetical variabilites in plant especially in those with limited genetic variabilities. It offers good prospects for the domestication of promising but underutilized wild species, for agricultural or horticultural uses as well as for improving adaptation of recently introduced crops to unsuitable environments (Anonymous, 1986). Mutagenesis has acquired popularity because of its simplicity, technical and economic viability, applicability to all plant species and usability at small or large scales (Siddiqui and Khan, 1999). More than 2000 plant varieties that contain induced mutations have been officially released for cultivation either directly as new varieties or used as parents to derive new varieties without the regulatory restrictions faced by genetically modified material (Maluszynski *et al*., 2000; Waugh *et al*., 2006). The prime strategy in mutation-based plant breeding has upgraded the well-adapted plant varieties by altering a desirable yield and quality of traitse oil content, malting content, size and quality of starch granules (Ahloowalia *et al*., 2004). In this investigation, the essential quality components of chilli such as Oleoresin, Capsanthin and Ascorbic acid were studied against the effect of mutagens such as Gamma rays and EMS.

## MATERIALS AND METHODS

### Mutagens employed

Both physical and chemical mutagens were used in this work to find out the variability in the genotype of chilli *Capsicum annuum* L. Var $K_1$. A physical mutagen, Gamma rays and Chemical mutagens Ethyl methane Sulphonate (EMS), were used as mutagenic agents.

**Gamma rays**

Gamma rays are one of the electromagnetic radiations, having low wavelength with high penetrable power. The source of gamma rays is $^{60}Co$, one of the labeled metals, which emit the rays. The irradiation was accomplished at Sugarcane Breeding Institute, Coimbatore, India.

**Ethyl methane sulphonate (EMS) ($CH_2SO_2OC_2H_5$)**

The chemical was obtained from HI-MEDIA Laboratories, Mumbai. It has a half-life period of 30 hours with a molecular weight of 124.16 and density of 1.20.

**Gamma rays**

Ten sets each of three hundred well-matured seeds chilli *Capsicum annuum* L. Var $K_1$ were taken for study. These set of seeds were packed in paper cover for irradiation with physical mutagen (gamma rays) and treated with 20, 30, 40, 50, 60 and 70 kR of gamma rays to determine the 50% lethal dose ($LD_{50}$) value. Irradiation was accomplished at Sugarcane Breeding Institute (ICAR), Coimbatore, India. The labeled cobalt ($^{60}Co$) was used as source of gamma rays. The irradiated seeds were repacked separately with wet paper and all the seeds in different doses were immediately placed on moist germinated paper in the Petri plates (9 × 3 cm size) separately in the laboratory condition. For each treatment 10 replicates were studied and the untreated seeds were germinated as control lines. $LD_{50}$ value was determined based on the lethality of the seedling ($15^{th}$ day) with 10 replicates in which 40kR of gamma rays showed 50% lethality.

**Ethyl methane Sulphonate (EMS)**

Five gram seeds of each treatment were presoaked for 12hrs in distilled water, blotted dry, and treated with 10, 20, 30, 40, 50 and 60mM of freshly prepared solutions of ethyl methane sulphonate for 4hrs with intermittent shaking at room temperature (28±2$^oC$). All seeds were uniformly exposed to EMS solution by stirring with a glass rod. After treatment, seeds were thoroughly washed in running water for 4 hrs to leach out the residual chemicals.

**Control**

Two sets of twenty five, dry, well matured, healthy and uniform in size of non-dormancy seeds were soaked in the double distilled water for 12 hours at room temperature (28±2$^{o}$C). These seeds were used as control (untreated seeds) and these were sown along with treated seeds.

**$M_2$ Generation**

The seeds harvested from $M_1$ generation were taken from individual treatments and used to raise $M_2$ generation plants. The $M_2$ generation was grown in complete randomized block design (CRBD) with five replications. All the recommended cultural practices namely, irrigation, weeding and plant protection methods were carried out during the plant growth period.

**Estimation of chlorophyll content (Arnon, 1949)**

0.5 mg of fresh leaf from the same plant of various conc. of $M_1$ and $M_2$ generation were ground in a pestle and mortar with 20 ml of 80 % acetone. The homogenate was centrifuged at 3000 rpm for 15 min. The supernatant was saved. The pellet was restricted with 5 ml of 80 % acetone each time, until it become colorless. All the supernatants were pooled and utilized for the determination of chlorophyll. Absorbance was measured at 645 and 663nm in spectrophotometer. The chlorophyll content was determined against acetone as standard using the following formulae:

Chlorophyll ‘a’ (mg/g fr. wt.) = (0.0127) × (OD663) - (0.00269) × (OD645)

Chlorophyll ‘b’ (mg/g fr. wt.) = (0.0229) × (OD645) - (0.00468) × (OD663)

Total chlorophyll (mg/g fr. wt.) = (0.0202) × (OD645) + (0.00802) × (OD663)

**Estimation of Capsaicin content (%) - Palacio (1977)**

The capsaicin content was estimated by procedure proposed by Palacio (1977). In this estimation procedure, 2 grams of ground-dried chilli was passed through No. 40 sieve (0.42 mm) and was placed in the 100 ml volumetric flask. The material was diluted with ethyl acetate up to 100 ml and allowed it to stand for 24 hours to extract. 1 ml of the extract was taken and diluted with 5 ml of ethyl acetate just before reading, and then 0.5 ml of vanadium oxytrichloride ($VoCl_3$)

solution (0.5% $VoCl_3$ in ethyl acetate) was added and the volumetric flask (100 ml) was shaken thoroughly and reading was taken at 720 nm. Then reading was subtracted from the value obtained with 0.5 ml $VoCl_3$ added to 5 ml ethyl acetate (blank) and the reading was compared with the standard curve prepared for capsaicin. The amount of capsaicin in the samples was expressed in percentage. The capsaicin content in fruits is expressed in terms of scoville heat units (Scoville, 1912). Suzuki *et al.,* (1989) have established the relationship that one per cent of pure capsaicin has a scoville heat value of 1, 50, 000.

$$\% \text{ capsaicin} = \frac{\text{Units mg capsaicin}}{1000 \times 1000} \times \frac{100}{1} \times \frac{100}{2}$$

**Oleoresin (ASTA Units)-A.O.A.C (1980)**

100 mg of powdered sample was transferred to 100 ml volumetric flask. The final volume was made up with acetone, shaken and allowed to stand for two minutes. 10 ml of extract was pipetted into another 100 ml volumetric flask and final volume made up with acetone and was shaken again. Absorbance of this solution was measured at 460mm against acetone as blank.

ASTA colour vale for oleoresin = $[(A_{ext} \text{ at } 460mm) \times 164 I_f]$/g sample

Where,

$$I_f \text{ (Correction factor)} = \frac{\text{Declared OD of NBS std. at 465 nm}}{\text{Observed OD of NBS std. at 465nm}}$$

Standard of NBS (National Board of Spice) is 1M Ferrous ammonium sulphate and declared OD is 0.64. In the spectronic, declared OD is equal to observed OD, there was no need to multiply with $I_f$.

**Estimation of Capsanthin/colouring matter (ASTA Units) – [A.O.A.C, 1980]**

100 mg of powdered sample was taken in 100 ml of volumetric flask, diluted to volume with acetone and corked tightly. The solution prepared was shaken well and allowed to stand in dark for sixteen hours at room temperature. The mixture was shaken again and particles were allowed to settle down for two minutes. A clear portion of the extract was transferred to cell and absorbance was measured at 465nm using acetone as blank.

$$\text{ASTA colour vale for capsanthin} = [(A_{ext} \text{ at } 465\text{nm}) \times (16.4\ I_f)]/\text{g sample}$$

**Estimation of Ascorbic acid content (Ranganna, 1979)**

5 ml of standard ascorbic acid solution was taken in a beaker and 5 ml of $HPO_3$ was added to it. This solution was titrated with the dye solution to a pink colour which persisted for 15 seconds. Dye factor (mg of ascorbic acid per ml of the dye) was determined by using the formula

$$\text{Dye factor} = \frac{0.5}{\text{Titre}}$$

Here,

0.5 means 0.5 mg of ascorbic acid in 5 ml of 100 ppm standard ascorbic acid solution

Titre = Volume of dye used to neutralize 5ml of 100 ppm standard ascorbic acid solutions along with 5 ml of metaphosphoric acid.

Ten grams of macerated sample was blended with 3 per cent metaphosphoric acid and the volume was finally made up to 100 ml. Out of this 100 ml solution, 10ml of solution was taken and titrated against 2,6-dichlorophenol indophenol dye till the appearance of rose pink colour. The results, thus obtained were expressed in terms of mg of ascorbic acid per 100g of sample.

The ascorbic acid content was calculated by using the following formula:

$$\text{Ascorbic acid (mg/100g)} = \frac{\text{Titer} \times \text{Dye factor} \times \text{volume made up}}{\text{Aliquot of extract} \times \text{Weight of sample taken for estimation}} \times 100$$

Here,

Titer = Volume of dye used to titrate the aliquot of extract of a given sample.

**$M_2$ Data Analysis**

The mean values for different characters in each treatment were calculated and expressed as percentage of increase and decrease over control. Analysis of variant methods was adopted for the statistical analysis of the data to find out the significant difference between the treatment and control.

**RESULTS**

**Chlorophyll content (mg/gfr.wt)**

Chlorophyll content was higher in 30mM EMS (0.148 mg/gfr.wt) than control (0.137 mg/gfr.wt) chilli plants. Besides $LD_{50}$ concentrations, other concentration showed slight increase in chlorophyll content. Whereas high concentrations showed minimum amount of chlorophyll content (Table-1).

**Capsaicin content (%)**

The mutagens influenced the capsaicin content with different levels of variation. In this generation, a little variation of capsaicin content was found at 40kR of Gamma rays (0.41 %) and 30mM EMS (0.43%) treatments than control plants (0.36 %) plants (Table-1).

**Oleoresin (ASTA units)**

Due to the influence of chemical mutagens, the oleoresin content was found to be higher in 30mM EMS (38.31 ASTA) followed by 40kR of Gamma rays (36.71 ASTA) treated plants. Above mentioned mean performance was observed to be slightly increased than control (36.14) plant (Table-2).

**Capsanthin content (ASTA units)**

A gradual increase of mean performance in capsanthin content was noticed with increasing concentration of mutagens up to certain concentration in $M_2$ generation than that of control. Among them, higher mean for capsanthin was observed at 30mM EMS (82.54 ASTA Units) followed by 40kR of Gamma rays (81.53 ASTA Units) in treated plants. This was significantly increased mean performance of capsanthin (colouring matter) than other concentrations and control (80.44 ASTA) (Table-2).

**Ascorbic acid (mg/100g)**

Ascorbic acid was higher in 30mM EMS (95.29mg/100g) followed by 40kR of Gamma rays (94.86) than control (92.70 mg/100g) plants. Whereas high concentrations of Ethyl methane sulphonate and Diethyl sulphate mutagens showed decreased quantity of ascorbic acid than control plants (93.47) (Table-3).

**DISCUSSION**

In $M_2$ generation, a significant difference in mean performance of capsaicin content in mutagenic treatments when compared to control. Capsaicin, the pungent principle of chilli was found to vary from (0.34-0.39 %) in EMS and Gamma rays treatments. This variation could probably due to the presence of gene modifying factors for pungency and the ratio of placental tissue to seed and pericarp Sreelathakumary (2000); Manju and Sreelathakumary (2002). Similarly, the local slightly pungent cultivar is 'Jeromin' with 78 mg/kg dry wt. of total capsaicinoids. It should be noted that crossing of local cultivars with Hungarian non-pungent ones (Szegedi17, Szegedi20, and Szegedi80) yield hybrids with light pungency. When Hungarian pungent cultivars are used as parentals (Szegedi178, Szegedi179, Szegedi411 and Kibedicsipos), the hybrids possess higher pungency. Jeromin × Szegedi178 and Kibedicsipos × Jariza with capsaicin content higher than 600 mg/kg dwt, and Jaranda × Szegedi179 with 495 mg/kgdwt, are the more pungent hybrids (Concepción Ayuso *et al.,* (2008).

Yoshiyuki *et al.,* (2010) investigated the hereditary pattern of capsinoid content, genetic analysis was conducted using $F_1$ and $F_2$ populations derived from crossing Himo and a pungent cultivar. Himo contained high levels of capsinoid, but capsaicinoid was not detectable. Pungent cultivars,

mainly contained capsaicinoids and produced capsinoids in trace amounts. Both Himo×No. 3446 $F_1$ and Himo×Yatsufusa $F_1$ plants mainly produce capsaicinoids, indicating the dominance of pungency and capsaicinoid biosynthesis. Of a total of 80Himo×No. 3446 $F_2$ plants, 59 plants mainly produced capsaicinoids and 21 produced capsinoids. This segregation ratio is consistent with the expected ratio of three capsinoid plant ($\chi 2 = 0.07$; $P = 0.79$) in chilli.

Foliar spray of Mepiquat chloride on bell pepper plants influences biochemical parameters *viz.*, total chlorophyll content, ascorbic acid and nitrate reductase activity and thereby increases fruit yield and quality. Ascorbic acid content of fruits from different treatments varied from 247.2 - 253.9 mg/100g fruit weight. Khadui *et al.*, (1987) showed that increased level of ascorbic acid content in Naphthalene Acetic Acid treated plants of Pusa Jwala variety of chilli over control with triple sprays of NAA (20 ppm). Also, the results of Tomlekova *et al.*, (2008) showed that the fruits are characterized by increased levels of β-carotene (provitamin A), oleoresin and capsanthin content in chilli pepper mutants. The results of the present study suggest that particular concentration of EMS treatment below the toxic level (*i.e.*, 30 mM EMS and 40kR of Gamma rays treated plants) can be used to increase the ascorbic acid content in *C. annuum*, which is the basis for any breeding program.

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