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ORIGINAL ARTICLE

# Estimation of genetic variability, mutagenic effectiveness and efficiency in $M_2$ flower mutant lines of *Capsicum annuum* L. treated with caffeine and their analysis through RAPD markers

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**Abstract** In the present investigation healthy and certified seeds of *Capsicum annuum* were treated with five concentrations of caffeine i.e. 0.10%, 0.25%, 0.50%, 0.75% and 1.0%. Germination percentage, plants survival and pollen fertility were decreased with the increase of caffeine concentrations. Similarly root length and shoot length were decreased as the concentrations increased in $M_1$ generation. Different mutants were isolated in $M_1$ generation. In $M_2$ generation, various flower mutants with changes in number of sepals, petals, anther size colour i.e. Trimerous, tetramerous, pentamerous with fused petals, hexamerous etc were segregated. Heptamerous and anther change was not observed in lower concentration viz. 0.1%. All these mutants showed significant changes in morphological characters and good breeding values at lower and intermediate concentrations. Mutagenic effectiveness and efficiency was observed on the basis of $M_2$ flower mutant frequency. It was generally decreased with the increase of mutagen concentrations. Cytological aberrations in mutants showed the decreasing trend at meiotic final stages. These mutants were further analysed through RAPD method and on the basis of appearance of polymorphic DNA bands, they distinguished these flower mutants genotypically. Among 93 bands 44 bands were polymorphic which showed great genetic variation produced by caffeine. As an

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outcome of that the above caffeine concentrations are good for the induction of genetic variability in *Capsicum* genotype.



## 1. Introduction

*Capsicum* ($2n = 24$, Family = Solanaceae) is an essential spice which is used in every Indian dish due to its pungency, flavour, aroma and colour. It also has various medicinal i.e. anti-inflammatory, analgesic, rubefacient, carminative and antioxidant (Sim and Sil, 2008), hypoglycaemic (Monsereenusorn, 1980), antifungal (De Lucca et al., 2006) and antimicrobial activities (Ribeiro et al., 2007). There are different morphological mutants, which have been isolated by various workers after the treatment of chemical mutagens in chilli (Raghuvanshi and Singh, 1982; Rostaino, 1983).

Mutation induction is an important and complementary method of plant breeding. At genic level mutation causes alterations in the structure and position of the gene on a chromosome then it is called as point mutation, which alter the phenotype of an organism. For any successful crop improvement programme, genetic variability plays an important role because it provides a spectrum of variants for effective and better selection which can be obtained using mutation, hybridization, recombination and selection processes (Dhumal and Bolbhat, 2012). Induced mutagenesis is an important and established method for plant improvement, where plant genes are altered by treated seeds and other plant parts with chemical mutagens (Sri Devi and Mullainathan, 2012). In any mutation breeding plan for the production of high frequency of desirable mutation, selection of an effective and efficient mutagen is indispensable (Roychowdhury and Tah, 2011). The application of molecular markers for the estimation of the variability of plant varieties and species is helpful in both detection of genetic relationships between them and making a system of plant genera, which involves the most important agricultural species (Sivolap et al., 2004). Caffeine (1,3,7-trimethylxanthine) is a purine alkaloid. It can interact with DNA and change the some of its physical properties (i.e. DNA denaturation temperature) and determine a higher rate of spontaneous mutations (Truta et al., 2007) and also inhibit DNA repair mechanism (Itoyama and Bicudo, 2000), causing DNA-DNA or DNA–protein linking (Amin, 2002). Due to its purine nature caffeine has mutagenic potential and it also has the ability to act synergistically in inducing chromosomal aberration in mammalian cells (Khursheed et al., 2009).

*Capsicum* has economic value so it is important to enlarge the genotypic and phenotypic variability, to observe some desired characters and to obtain new valuable genotypes through different methods, including chemical mutagenesis. For this reason, the present experiment was designed to report the genetic variability in $M_2$ flower mutant lines in chilli which was isolated through the mutagenic effect of caffeine. For variability we used morphological characteristics, meiotic behaviour of mutants and their molecular analysis because it is a robust and reliable method to detect interspecific genetic variability.

## 2. Materials and methods

### 2.1. Experimental plan and procedure

Fresh, healthy and certified seeds of *Capsicum annuum* L. were collected from IARI-Pusa campus New Delhi. Seeds were pre-soaked in distilled water and 24 h after pre-soaking they were treated with five concentrations i.e. 0.10%, 0.25%, 0.50%, 0.75% and 1.0% of caffeine (issued from departmental chemical store) solution (pH4) prepared in phosphate buffer (6 h) along with one control set pre-soaked in distilled water in $M_1$ generation. Now treated seeds were sown immediately to raise $M_1$ generation. The desirable variants from $M_1$ generation were selected on the basis of their phenotypical features and harvested separately for the calculation of frequency, meiotic aberrations, mutagen effectiveness and DNA damage in the next generation ($M_2$). Flower variants were scored and classified.

### 2.2. Growth parameters

The experiments were done to determine the effect of caffeine on germination, survival, pollen fertility, root and shoot lengths in $M_1$ generation. Each treatment was replicated five times and for each replication 50 seeds were sown and tested for all growth parameters. Effect on root and shoot was measured in terms of length of root and shoot respectively for 30 days and results recorded from randomly selected seedlings of each replicate. Some seedlings were transferred into pots and after maturity seeds with identified variants for $M_2$ generation in all the concentrations were harvested.

### 2.3. Methodology of $M_2$ flower mutant selection

$M_1$ generation seeds were harvested and pre-soaked in distilled water (24 h), planted to raise the $M_2$ generation in control and isolated mutants were also sown individually. In $M_2$ generation, various flower mutants were identified with different morphological traits such as growth, plant height, number of fruits, No. of seed, number of sepals, petals and anthers colours in all the concentrations and they were harvested separately.

#### 2.3.1. Mutation effectiveness and efficiency

Mutation frequency was calculated as $M_2$ plant percentage while mutation effectiveness and efficiency were calculated on the basis of Konzak et al. (1965) formula.

$$\text{Mutagenic effectiveness} = \frac{\text{Mp}}{\text{Conc.} \times \text{duration}}$$

$$\text{Mutagenic efficiency} = \frac{\text{Mp}}{\text{I or S}}$$