

# Physical and chemical induced mutagenesis study for identifying lethality dose in chick pea (*Cicer arietinum* L.) Var. Co – 4

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## ABSTRACT

The present study was conducted in order to determine the effect of gamma rays and EMS on seed germination, Seedling height and root length in chick pea to identify the lethal dose (LD<sub>50</sub>). In this regard, the healthy seeds of chick pea was subjected to different doses/concentrations of gamma rays (20, 30, 40, 50 and 60kR) and EMS (10, 20, 30, 40 and 50mM) for inducing mutation. The effect of gamma rays and EMS was determined by measuring the seed germination, seedling height and root length under the conditions of the M<sub>1</sub> generation. The results shows that, the seed germination, seedling height and root length were significantly decreased with increasing doses/concentrations. The LD<sub>50</sub> values were observed based on the growth reduction of seedlings after treatments with mutagen. The effective doses/concentrations which caused 50% growth reduction were observed in 40kR in gamma rays and 30mM in EMS.

**Keywords:** Germination percentage; Seedling height; Root length; LD<sub>50</sub>

## 1. INTRODUCTION

Chick pea (*Cicer arietinum* L.) is said to be one of the oldest pulse crop known and cultivated from ancient times both in Asia and Europe. India is one of the major chick pea producing country in the world breeding methods. A critical appraisal of all the chick pea breeding work in our country and at global scale indicates that like other grain legumes, little concentrated efforts have been made for its genetic amelioration. The hybridization work undertaken is also very limited and it is probably due to the tedious crossing procedure because off small and delicate flowers. Induced mutagenesis can play an important role in renewal as well as augmentation of the natural variability to some extent. Little natural variability in chick pea for conspicuous morphology and physiological characters, several workers have attempted for induction of mutation using either physical or chemical mutagen for new genotypes.



**Photo 1.** *Cicer arietinum* L.

## 2. MATERIALS AND METHODS

The CO – 4 variety of chick pea was taken for the mutation studies. Healthy seeds were treated separately with chemical (EMS) and physical (gamma rays) mutagens. For chemical mutagenic treatment, seeds were presoaked in distilled water for 6 hours followed by treatment in freshly prepared solutions mutagens for 6 hours. EMS solution of 10, 20, 30, 40 and 50mM was prepared in 1.0 M phosphate buffer at pH -7. The chemical mutagenic treatments were given at  $27 \pm 2$  °C with intermediate shaking. The seeds were thoroughly washed under running tap water to terminate the reaction of the chemical before sowing in the field. For the physical treatments, dry seeds were irradiated with gamma irradiation dose of 20, 30, 40, 50 and 60kR from a  $^{60}\text{Co}$  source from IGCAR, Kalpakkam. The treated seeds were sown in the field along with control to raise the  $M_1$  generation at the Botanical Garden, Department of Botany, Annamalai University in a complete Randomized Bloch Design (CRBD).

The germination of seeds was observed on 5<sup>th</sup> day and 15<sup>th</sup> day. The seedling height and root length of the plants were measured after two weeks. The data related data about seedling height, root length and per cent of germination were collected and recorded. The statistical analysis was carried out by using NPROC software.

### 3. RESULTS

#### 3.1. Effect of gamma rays and EMS on germination

Germination seeds were observed and the emergence cotyledonary leaf was taken as the indication of germination. Generally a gradual reduction of germination was noted among the various dose/concentrations studied. Among them lowest doses/concentrations of treatments shows highest germination percentage. The percentage shows a dose dependant decrease while increasing the concentrations (Table 1, Fig. 1).

#### 3.2. Effect of gamma rays radiations on seedling height and root length

Analysis of average seedling height and root length was observed on 15<sup>th</sup> and tabulated (Table 2, Figure 2) shows that, the seedling height decreased in proportion while increasing in the doses/concentrations of mutagen. The highest length of seedling was observed in 20kR of gamma (11.47) and 10mM of EMS (11.20). Based on Table 2, the maximum reduce in seedling height was observed, when it was exposed to higher doses gamma rays (7.56) and EMS (6.30).

The data on root length were shows a decreasing tendency with increasing doses/concentrations of mutagen. The maximum root length was found in control (9.93). In treated populations, maximum root length was observed in lower doses/concentrations such as 20kR in gamma rays and 10mm in EMS (Table 2)

### 4. DISCUSSION

Seed germination is an important parameter to estimate the effect of mutagen on plants. Inhibition in seed germination, after the treatment of seed with different mutagen is a convenient technique for studying their effects of mutagens on plants.

The results indicate that, per cent seed germination decreased with increasing dose/concentration of gamma rays and EMS in chick pea. This clearly indicates that the mutagen have exerted an inhibitory effect on seed germination. Such a dose dependant inhibition of germination was reported earlier by Athwal (1963) in *Cicer*, Ganesan (1998) in *Sesame*, Kumar & Mishra (2004) in Okra, Haque fazal et al., (1981) and Chaturvedi et al., (1982) in pigeon pea.

Reduction in germination in mutagenic treatments has been explained due to delay or inhibition of physiological and biological processes necessary for seed germination, which include enzyme activity (Kurobane et al., 1971), hormonal imbalances (Chrispels and Varnel, 1967) and inhibition of mitotic process (Ananthaswamy et al., 1971). The inhibition of seed germination percent was more in EMS when compared to the gamma rays treatments. This result supports the earlier works done by Dhakshanamoorthy et al., (2010) in *Jatropha*, Sagade and Apparao (2011) in *Vigna* and Dubey et al., (2011) in *Cymopsis*. The presoaking of seeds

increases the sensitivity to chemical mutagens. This subjected to various reasons such as change in metabolic processes (Sharma, 1969). The maximum reduction in percent seed germination in chick pea caused by EMS might be due to the change in metabolic conditions of the cells during pre soaking. Both gamma rays and EMS are potent mutagens well known for their action in inducing point mutations, enzyme inhibition and chromosomal aberrations.

Effect of mutagens on root length and seedling height to identify the biological influences of different physical and chemical mutagens in M<sub>1</sub>, seedling height is mostly utilized as an index (Nadeau and Frankel, 2000).

The seedlings raised from the treated seeds show a decreased trend, from lower to higher dose of mutagen in root and shoot length. It could be due to the uneven damage to the meristematic cells as a consequence of genetic injury. The badly damaged cells would produce only a few cell progeny and growth will recur from those cells which are genetically least damaged.

The reduction in plant height by chemical and physical mutagens was ascribed to different factor. According to Markeen kousar et al., (2007) reduction seedling growth with higher doses of gamma rays, may be due to the gross injury caused at cellular level either due to gene controlled biochemical process on acute chromosomal aberration or both. Gunckel and Sparrow (1962) indicated that the genetic material of the cell is certainly sensitive to radiation damage, but both primary and secondary physiological effects may be responsible for many changes. According to Sax (1963) the mutagen might inactivate the meristem and cause hormonal disturbances leading to reduction in plant height.

## 5. CONCLUSION

From the study, it can be concluded that, a dose dependent decreasing tendency was observed in germination percentage, seedling height and root length. In both treatments, maximum reduction was observed in higher doses/concentrations, and EMS caused more damage than the gamma rays. Also the LD<sub>50</sub> value was observed in 40kR of gamma rays and 30mM of EMS.

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