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STUDIES ON EXPERIMENTAL MUTAGENESIS ON CHICK PEA (*CICER ARIETINUM* L.) INDUCED BY ULTRAVIOLET RAYS AND ETHYL METHANE SULPHONATE

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ABSTRACT
Genetic variability is the most essential prerequisite for successful crop improvement programme as it provides spectrum of variants for the effective selection, which can be achieved through the processes of mutation. Induced mutagenesis through physical and chemical mutagens is an important and sophisticated approach to induce desirable variability, resulting in the creation of new varieties with better characteristic. In the present study, seeds of chick pea (***Cicer arietinum*** (L.) ICCV-2 was mutagenized with different time intervals /concentrati ons of UV rays and EMS to determine the effect of mutagens on different quantitative characters. The quantitative characters that were analyzed in M3 generations were included: days to first flower, plant height, number of branches per plant, number of leaves per plant, number of pods per plant, pod length (cm), pod breadth (cm), pod yield per plant (g) and twenty seed weight (g). The results showed that in M3 generation, 1.0% of EMS and 5minutes intervals' of time of UV rays showed a significant increase in all the quantitative characters when compared to control and other doses/concentrations. However, consistent decrease in the flowering period was found with decrease in the mutagenic concentration. We conclude that the mutagenic effect of UV rays and EMS were found to be beneficial in improving the growth and yield of Chick Pea.

KEYWORDS: UV rays, EMS, Chick pea, Induced mutation.

INTRODUCTION
Legumes are one of the most important groups of crop plants and have been the subject of efforts to improve desirable traits including their *in vitro* culture response. Since legumes are notoriously recalcitrant to regenerate from tissue culture, much effort has been devoted to developing and optimizing efficient *in vitro* regeneration systems to facilitate a variety of technologies (Polanco and Ruiz, 1997). The ability to regenerate plants from cultured cells, tissues or organs constitutes the basis of producing transgenic crops. Successful regeneration of legume species has been greatly aided by species-specific determination of critical parameters, such as explants source, genotype and media constituents. (Parrot *et al.*, 1997).

Chickpea (*Cicer arietinum* L.), commonly called gram, Bengal gram, or garbanzo bean, is the most important food grain legume of South Asia and the third most important in the world after common bean (*Phaseolus vulgaris* L.) and field pea (*Pisum sativum* L.). Chickpea is a diploid with 2n=2x=l6 chromosomes and a genome size of approximately 750 Mbp (Arumuganathan and Earle, 1991). Chickpea is one of the first grain crops cultivated by man and has been uncovered in Middle Eastern archaeological sites dated to the eighth millennium BC (Zohary and Hopf, 2000). Two distinct market type classes, desi and kabuli, are recognized in chickpea (Pundir, Rao, and van der Maesen, 1985). The desi types that account for about 85% of chickpea area usually have small, angular shaped, dark-colored seeds with a rough surface, pink flower, anthocyanin pigmentation on the stems, and either semi-erect or semi-spreading growth habit. The kabuli types, which cover the remaining 15% area, usually have large "rams head"-shaped smooth surface seeds, lack of anthocyanin pigmentation, and semi-spreading growth habit. It has become increasingly clear during the last few decades that meeting the food needs of the world's growing population depends, to a large extent, on the conservation and use of the world's remaining plant genetic resources. Conservation without use has little point and use will not come without evaluation. Genetic resources encompass all forms of the cultivated species,

as well as their related wild species (Harlan, 1984). That is a general concept to which chickpea is no exception. In reviewing genetic resources and their multifaceted applications in chickpea genetic improvement, we have placed more emphasis on the wild genetic resources of the cultivated chickpea, while providing a brief overview of resources available in the cultivated species.

Induced mutations are considered as an alternative to naturally occurring genetic variation that serves as the source of germplasm for crop improvement programmes and also as an alternative to hybridization and recombination in plant breeding. It has the potential to create genetic variability in quantitatively inherited traits in various crop plants within the shortest possible time3-7. Many new cultivars have been directly or indirectly released in the world through induced mutations. Mutation breeding is an established method for affecting genes either by treating seeds or other plant parts through chemical and physical mutagens8. It has played a productive role in sustainable agriculture. The main advantage of mutation breeding is the potential to improve one or two characters without altering the original genetic make- up of the cultivar. Using mutation breeding, genetic improvement of any yield attributes either qualitative or quantitative trait, has been successfully achieved in crops. Furthermore, mutation breeding requires less time to develop crop cultivars as compared to the conventional breeding (Bolbhat and Dhumal 2009 and Manjaya. 2009). Physial mutagens namely X- rays, gamma rays, fast neutrons, thermal neutrons, ultraviolet and beta radiations have been used for induced mutagenesis (Yaqoob and Rashid 2001). Apart from physical mutagens, several chemical mutagens were also frequently used for induced mutagenesis in crops, respectively, Ethyl Methane Sulphonate (EMS), Methyl Nitroso Urea (MNU), N-nitroso-N-methyl Urea (NMU) and Ethyl Nitroso Urea (ENU). These mutagens have been used for the induction of useful mutants in a number of crop plants (Makeen *et.,al* 2013). Gamma rays are the most energetic form of electromagnetic radiation, having the energy level from around 10 kilo electron volts (keV) to several hundred keV. Therefore, they are more penetrating than other types of radiation such as alpha and beta rays (Kovacs and Keresztes, 2002). Gamma sources are used to irradiate a wide range of plant materials, like seeds, whole plants, plant parts, flowers, anthers and pollen grains etc.

Chickpea has one of the highest nutritional compositions of any dry edible grain legume and does not contain significant quantities of any specific major antinutritional factors. On an average, chickpea seed contains 23% of highly digestible protein, 64% total carbohydrates, 47% starch, 5% fat (primarily linoleic and oleic acids), 6% crude fiber, 6% soluble sugar, and 3% ash. The mineral component is high in phosphorus (343 mg/100 g), calcium (186 mg/100 g), magnesium (141 mg/100 g), iron (7 mg/100 g), and zinc (3 mg/100 g) (Williams and Singh, 1987). Used in a variety of ways, chickpea is not only good for human health but also for soil health. It meets 80% of its nitrogen (N) requirement from a symbiotic rhizobial interaction, which enables the crop to fix up to 140 kg N haí1 from atmosphere (Saraf *et., al* 1998). It leaves substantial amount of residual nitrogen behind for subsequent crops and adds much needed organic matter to maintain and improve soil health, long-term fertility, and sustainability of the ecosystems. In recent years, chickpea has also gained popularity in broad-acre cropping systems in developed countries, particularly Australia and Canada (Siddique and Sykes, 1997). A dryland crop requiring minimal inputs, chickpea is a boon to the resource-poor marginal farmers in the tropics.

Role of pulses in Indian agriculture needs hardly any emphasis; India is a premier pulse growing country. The pulses are the integral part of the cropping systems of the farmers all over the country because these crops fit in well in the crop rotation and crop mixtures followed by them. Pulses are important constituents of the Indian diet and supply major part of the protein requirements. Pulse crops, besides being rich in protein and some of the essential amino acids, enrich the soil through symbolic nitrogen fixation from atmosphere.

In India, the total food production in 1999-2000 was about 209 million tones, out of this only 13.4 million tones was contributed by pulses. The production of cereals increased by 460 per cent since 1950-51 the production of pulses has increased only 178 per cent. There is a shortage of pulses in the country. The price has increased considerably and the consumer is hard hit to buy his requirements. Thus, the availability of pulse per capita per day has proportionately declined from 71 g (1955) to 36.9 g (1998) against the minimum requirement of 70 g per capita per day. There is not much possibility of the import of pulses in the country. The production of pulses has to be increased internally to meet the demand.

Gram commonly known as 'chick pea' or Bengal gram is the most important pulse crop in India. Chick pea occupies about 38 per cent of area under pulses and contributes about 50 per cent of the total pulse production of India. It is used for human consumption as well as for feeding to animals. It is eaten both whole fried or boiled and salted or more generally in the form of split pulse which is cooked and eaten. Both husks and bits of the 'dal' are valuable cattle feed. Fresh green leaves are used as vegetable (sag). Straw of chick pea is an excellent fodder for cattle. The grains are also used as vegetable (chhole). Chick pea flour (besan) is used in the preparation of various types of sweets. Chick pea is considered to have medicinal effects and it is used for blood purification. Chick pea contains 21.1 per cent protein, 61.5 per cent carbohydrates, 4.5 per cent fat. It is rich in calcium, iron and niacin.

The cultivated chickpea species has been taxonomically placed in the genus Cicer, which belongs to the family Fabaceae and its monogeneric tribe Cicereae Alef. (Kupicha, 1981). Presently, the genus Cicer consists of 43 species, divided into 4 sections, Monocicer, Chamaecicer, Polycicer, and Acanthocicer, based on their morphological characteristics, life cycle, and geographical distribution (Van der Maesen, 1987) of these Cicer species, sharing the annual growth habit with chickpea, are of particular interest to breeders. Of the nine annual Cicer species, eight are classified within the Monocicer section, and one, C. chorassanicum, within the Chamaecicer section (Kazan and Muehlbauer, 1991; Muehlbauer, Kaiser, and Simon, 1994). Thirty-three of the remainder species are known to be perennial while *C. laetum* Rass. & Sharip has an unspecified life cycle (Van der Maesen, 1987) Annual Species

It acts on genetic material by ionization leading to more of chromosomal rather than point mutations. When a biological material is irradiated, a gamma rays photon hits an orbital electron of the atom, the electron get excited and in turn ejected out leaving behind a positively charge atom. The ejected electron has tremendous energy and is capable of causing further ionization along its path leading to mutations through change in nucleotide sequences (Bind, *et.,al* 2016). Gamma irradiation has provided number of useful mutants and still shows an elevated potential for improving vegetative plants. It has been employed for the development of 64% of the mutant varieties (Souframanien *et. al* 2002). EMS is one of the most frequently used alkylating agents for chemical mutagenesis in plants due to its potency and ease with which it can be used (Wattoo *et.,al* 2013). EMS selectively alkylates guanine bases causing the DNA-polymerase to favor placing a thymine residue over a cytosine residue opposite to the O-6-ethyl guanine during DNA replication, which results in a random point mutation. A majority of the changes (70-99%) in EMS mutated populations are GC to AT base pair transitions (Sikora *et. al* 2011 and Rafi *et. al* 2016). EMS is now being widely accepted as a powerful mutagen and is used commonly in the induction of mutations in various crop plants because of its ability to induce a higher frequency of mutations in higher plants (Ahirwar *et. al* 2014). Mutagenic agents have been used to induce useful phenotypic variations in plants for more than seventy decades. So far, 3222 number of crop varieties have been developed through induced mutagenesis in varieties of crop such as cereals, oilseeds, pulses, vegetables, fruits, fibres and ornamental plants. Against this background, the aim of the present investigation was to study the effect of gamma rays and Ethyl Methane Sulphonate on different quantitative characters of Chickpea (*Cicer arietinum* L.).

MATERIALS AND METHODS

Experimental plant material selected for the present investigation was Chickpea (*Cicer arietinum* L.) ICCV-2 the seeds of this variety were procured from ICRISAT Hyderabad, Telangana, India.

Mutagens employed: A physical mutagen, UV rays and a chemical mutagen, Ethyl Methane Sulphonate (EMS) were used at various intervals of time /concentrations to induce mutation in the selected plant material and to achieve genetic variability.

Physical mutagen: UV rays treatment: UV rays are electromagnetic radiations, having shorter wave length with high penetrable power. The source of UV radiation used in the present investigation. The soaked seeds of Chickpea (*Cicer arietinum* L.) variety ICCV-2 were soaked in Distilled water for 24 hours 20 seeds in Petri dish contains water for irradiation and treated with 1.0 min, 2min, 3min, 4min and 5minutes of UV rays. The radiation was accomplished at under the laminar air Flow Department of Biotechnogy Karimnagar, Telangana, India. The irradiated seeds were repacked separately with wet paper and the seeds from different time intervals were immediately placed on absorbent cotton-wet Petri plates (9×3 cm size) separately in the laboratory condition. For each treatment, 20 replicates were studied and the untreated seeds germinated were used as control lines. Based on the reduction of 50 percent germination, the LD50 value was determined. Five treatments of UV rays around LD50 value were fixed for further studies.

Chemical mutagen: Ethyl Methane Sulphonate treatment: MS ($CH_3SO_3C_2H_5$), a mono functional alkylating agent was obtained from HI-MEDIA laboratories, Mumbai. Healthy, dry seeds were pre-soaked for 6 hours in distilled water and treated with EMS at 0.1, 0.2, 0.3, 0.4, 0.5% (v/v) concentration along with untreated control treatment. The seeds were further incubated at room temperature for 3 h in a shaker with mild shaking (45 rpm). After EMS treatment, the seeds were thoroughly washed three times with running tap water and air-dried. All the seeds were uniformly exposed to EMS solution by stirring with a glass rod.

The treated and untreated Chick pea seeds were grown in polythene bags (10 seeds/bag) in randomized three replicates of 10 seeds (n = 30). Data were collected on germination percentage after 10 days. When M1 plants became 20 days old, they were transferred to soil pots and routinely irrigated. Data were collected for: seed germination, plant height, number of monopodial branches, number of sympodial branches, number of bolls/plant, boll weight, seed chick pea weight and lint percentage.

Control: Untreated dry seeds presoaked in distilled water for 6 hours were used as control.

Raising of M3 generation: In order to raise the M3 generation, the treated seeds (UV rays and EMS) along with control were sown in a randomized block design with a spacing of 30cm between rows and 15cm between

seeds at the Botanical Garden, S.R.R.College Karimnagar. The seeds of M2 generation, collected from fifteen randomly selected plants of all the treatments and control were used to raise the M3 generation. All the recommended cultural measures namely, irrigation, weeding and plant protection methods were carried out during the growth period of the crop.

Studies of Quantitative characters (M3generations): The quantitative characters observed in M3 generation were analyzed. The quantitative characters are as follows.

Plant Height (cm): The height of the plant was measured at the time of final harvest from the ground level to the growing tip of the plant using metre scale and expressed in cm.

Numbers of Branches: Total number of primary branches produced by an individual plant were counted and recorded.

Days to first flower (days): Number of days taken by the plant from sowing to anthesis of first flower.

Number of Leaves per plant: Total number of leaves at maturity was counted and recorded as the number of leaves per plants.

Number of pods per plant: The number of pods obtained from the plant were counted and recorded.

Pod length (cm): The length of the pod was measured and expressed in cm.

Pod yield per plant (g): Pod yield per plant was worked out by using digital electronic balance and expressed in grams.

Pod breadth (cm): The breadth of the pod was measured and expressed in cm.

Hundred Seed weight (g):100 Seed weight was worked out by using digital electronic balance and expressed in grams.

RESULTS

The results of analyses of variance indicated that mutagen, dosage and treatment period significantly influenced Chick pea seed germination percentage, number of leaves, radicle length and seedling height (Table 1). Pre-treatment significantly affected only germination rate and radicle. Among the first-order interactions, mutagen × treatment period and dosage × treatment period were significant for all the traits. For seedling height, second and third-order interactions were significant. All the main effects (mutagen, dosage, treatment period) along with first and second-order interactions were highly significant for seed viability.

Table 1: Analysis of variance on 30 days to above 50% emergence, germination percentage Radical length seedling height and Number of leaves among *Cicer* ICCV-2 variety when tested using Five UV Radiation and EMS Time /Concentration at three temperature regime and four exposure time.

Source of UV Rays	% of Germination	Radical length	Seedling height	Number of Leaves
1 Min	75	3.0 cm	30.0 cm	**25**
2 Min	80	2.8 cm	26.0 cm	26
3 Min	76	2.0 cm	20.0 cm	20
4.Min	60	1.5 cm	18.0 cm	19
5.Min	50	1.0 cm	16.0 cm	17
Control	95	3.8 cm	17.0 cm	15
Source of EMS				
0.5	65	1.8 cm	26.0 cm	32
1.0	58	1.0 cm	23.0 cm	25
1.5	45	0.8 cm	20.0 cm	24
2.0	35	0.5 cm	16.0 cm	20
2.5	30	0.3 cm	15.0 cm	10
3.0	25	0.2 cm	12.0 cm	09
Control	94	3.7 cm	17.5 cm	16

Ethyl methane sulfonate

Average germination percentage reduced with increasing mutagen concentration and treatment period where germination percentage was reduced from 94.0% in the control to 30% in the treatment with 3.0% EMS (Table 1). This trait was also reduced by increasing treatment period from 2 to 4 h where the germination percentage changed from 30.0% in presoaked seeds treated for 3 h to 6% in presoaked seeds treated for 10h with EMS. The treatment with 3.0% EMS acting similar to those of 10 h treatment with different concentrations of this mutagen almost blocked seed germination. The highest germination rate index (65) belonged to the presoaked control treatment and the least amount was related to the 5 h treatment with 0.5% of EMS in of no presoaked seeds (Table 1). Seedling height and radicle length also

decreased with increasing EMS concentration and treatment period (Tables-1). Pre-soaking did not significantly alter seedling height and radicle length traits. In both pretreatment conditions, treatment periods higher than 6 h affected neither the germination rate nor the seedling height of EMS-treated seeds. Non-presoaked seeds performed superior than presoaked ones in most of the treatments.

Showing the treated EMS (Chemical) & UV (Physical) Radiated Chick pea seeds Germinated in the bottles

DISCUSSION

Flowering plants are particularly well adapted to random mutagenesis because large, saturated mutant populations can be generated through chemical mutagenesis. Such populations can then be screened for the particular phenotypes using 'reverse screened' tools, which are conducted based on gene sequence for mutations in the target gene (Stephenson *et. al.* 2010). It is important, therefore, to determine the level of mutagen treatment necessary to achieve the utmost mutation load in an important oilseed crop species such as canola. The interdependence of treatment variables that influence the degree of M1 seed lethality induced by a mutagen is clearly illustrated by the interactions between mutagen concentration, treatment period and pretreatment observed in this study. When one considers that these are only a few of the treatment variables that could have been investigated, it becomes even more apparent that the reaction of mutagen with the cellular constituents is complex, underscoring the necessity for close control of experimental conditions to ensure repeatable treatment effects (Fowler and Stefansson, 1972). In this study, inverse relations were found between mutagen concentration and both rate and percentage of M1 seed germination in Chick Pea. These results are in agreement with the findings of previous research with other plants (Afsar *et., al.* 1980; Fowler and Stefansson, 1972; Padavai and Dhanavel, 2004; Singh and Kole, 2005). In the case of EMS, treatments with 1.0% for 4 h pretreatment conditions (Table 1). Fowler and Stefansson (1972) reported that increasing of EMS concentration from 0 to 1.0% adversely affected germination percentage. The interaction between dosage and duration of treatment for germination percentage was significant. This result shows the importance of duration of mutagen treatment in finding an optimal mutagenic dose. Pretreatment had no significant effects on traits in most of the treatments in this study. Soaking increases mutagen penetration into seeds and leads to higher metabolic activities, but there would be no need for presoaking if the duration of treatment with mutagen is long enough (Fowler and Stefansson, 1972).

This is because at higher concentrations of the mutagen, some mutants were eliminated from the population in the first generation, or they became sterile if they did survive. This is due to mutagenic effects on plant genes and/or chromosomal aberrations. The extent of reduction in growth is related to the mechanism of action for a given mutagen. Mutagens may inhibit an energy supply system resulting in the inhibition of mitosis which can be associated with seedling growth depression. Seed's physiological conditions during treatment greatly influence the magnitude of growth depression (Afsar *et., al.* 1980). Thus, breeders are interested in finding a mutagenic dose that induces adequate mutagenic outcome but which results in low sterility and lethality. Efficiency of the LD50 criterion has been validated by almost all researchers (Das and Haque, 1997; Gustafson, 1989; Hu and Rutger, 1992; Snustad and Simmons, 2006). According to this criterion, treatment with 0.8% EMS solution for 6 h has led to 50% lethality compared to that of control. Nevertheless, this mutagenic treatment may be proposed as the appropriate treatment conditions when one considers overall genomic aberrations caused by a higher mutagenic dose. Jabeen and Mirza (2004) subjected *Capsicum annum* seeds to different treatment levels of EMS (0.01, 0.1 and 0.5%) and two durations of exposure (3 and 6 h) and suggested that using 0.5% EMS for 3 h could induce appropriate morphological mutations. Das and Haque (1997) also studied the responses of sesame seeds to gamma rays and EMS in M1 generation. In their study, the optimum dosages for mutation induction were 0.7 to 0.9% EMS as determined by the LD50 criterion. The optimum dosage of EMS for rice was 8 h treatment with 1% EMS according to Padma and Reddy (1977). Compared to the control, treatment with the 12 mM ENU solution for eight hours and non-soaking pre-treatment induced 50% reduction in germination percentage in canola seeds and this treatment would, hence, is an optimal dose of ENU in

mutagenic studies. In the case of NMU, treatments of seeds with the 9 mM solution for 8 h could be proposed for enhancing the mutagen efficiency. This finding also confirms the earlier results of Ramulu (1972) with sorghum who observed that lower dosages of NMU are more efficient than higher concentrations. On one hand, application of lower mutagen concentrations is safer because it causes less sterility and abnormalities. From a breeding point of view, however, application of higher mutagen concentrations results in the higher frequency of induced mutations.

The mutagenic effect was showed a decline in the days to first flower at 1 Min of UV rays treatment than other treatments and control.

CONCLUSION

Mutation induction has proven to be a workable, sustainable, highly efficient, environmentally acceptable, flexible, non-hazardous and a low cost technology in the breeder's toolbox to enhance crop improvement. The present investigation was carried out to study the effect of gamma rays and Ethyl Methane Sulphonate on mean performances of some quantitative characters. In M3 generation, the quantitative characters were improved by gamma rays and EMS treatments. Among the various conc./dose of mutagenic treatments, 30mM of EMS and25KR of gamma rays treatment resulted higher genetic variability. Overall, the results of the present study indicate that gamma irradiation and EMS treatment induce genetic variability in Garden bean.

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