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Historical Development of Genetic Divergence by Radiomimetic agents

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ABSTRACT: The radiomimetic agents like, EMS (Ethyl Methane Sulphonate), MMS (Methyl Methane Sulphonate) and MES (Methyl Ethane Sulphonate) induce physiological and genetic changes in the plants. Mutagens have remarkable possibilities of improving plants with regard to their quantitative as well as qualitative characters. As a result of progressive in understanding the role of induced mutations, a number of economically useful mutant varieties have been commercially released. A rapid growth in the field of genetic divergence by Radiomimetic Agents has been witnessed in the late era of twentieth century that has bought revolution in the agricultural productivity. The paper reviews the historical development of genetic divergence by radiomimetic agents.

Keywords: Mutagens, genetic divergence, historical development, radiomimetic agents,

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INTRODUCTION

An understanding of nature and magnitude on existing genetic variability to the available germplasm for important yield contributing characters is necessary for successful breeding programme. The selection for yield generally remains unsuccessful in achieving desirable results, because yield is dependent on its component characters. Therefore, the knowledge of association among yield and its component traits are important for developing a suitable selection strategy. Yield being a complex character requires an efficient breeding programme to achieve the desired genetic improvement for the genetic architecture of yield must be thoroughly understood.

The usefulness of any mutagen in plant breeding not only on its effectiveness, but also upon its efficiency. Mutagenic effectiveness is a measure of the frequency of mutation induced by unit mutagen dose, whereas mutagenic efficiency is the measure of proportion of mutation in relation to undesirable changes like lethality, sterility and meiotic aberrations.

Mutation breeding has been very useful in inducing variability and become a potential tool in any plant breeding programme in the changing agriculture pattern while radiomimetic agents like, Ethyl methane sulphonate (EMS), Methyl methane sulphonate (MMS) and Methyl ethane sulphonate (MES) are found equally to ionizing radiation or even many more times effective and efficient mutagens. The relative ease of application and comparatively low cost of chemical mutagens has caused an unprecedented argue of interest in the artificial induction of mutation with the radiomimetic chemical agents. On these basic genetic information of fenugreek (*Trigonella foenum- graecum* L.) is scanty.

Fenugreek (*Trigonella foenum-graecum* L.) is a multi-purpose annual autogamous crop grown as spice, fodder and also for vegetable crops as leafy vegetable belongs to family Fabaceae (Bentham and Hooker (1862-1883). The seeds and leaves are rich source of vitamin A, vitamin C, protein, carbohydrates and minerals especially organic iron, phosphorus and calcium etc. The seeds of fenugreek contain alkaloid, Colin, bitter material, fatty acid, diastase which are excellent remedies for dysiorexia and weakness resulting from emaciation. It improves the appetite, increase the number of red blood cells. Very recently, it is uses as flavouring media in bakery and become very popular (Makai *et al.*, 2004). Fournier (1972), Paris *et al.* (1975), Sauvaire *et al.* (1976), and Abdul-Barry *et al.* (2000) has confirmed the antidiabetic actions of Fenugreek. Seeds of *Trigonella foenum-graecum* L. are used in the indigenous system of medicine (Chopra *et al*., 1966).

STUDIES ON GENETIC DIVERGENCE

A. Different Mutagens, effects and crops

The importance of micromutations in evaluation was stressed by Baur (1924). Stadler (1928, 1930) demonstrated mutagenic effect of radiations and radium on plants of barley and maize. Later the work was preceded by Stubbe and Wettstein (1941). Auerbach and Robson (1942) in Britain and Ochlkers (1943) in Germany independently reported the radiomimetic effects of certain chemicals. It was subsequently discovered that there are several types of ionizing radiations capable of inducing mutations such as X- rays, gamma rays, protons, neutrons, alpha and beta particles, which may be utilized for inducing mutations. Out of these, gamma rays have been most extensively employed for the obvious reason that they have shorter wavelength therefore possesses more energy per photon than X-rays and other radiations. However, Rapoport (1948) reported that out of all the chemical mutagens so for reported the nitroso compounds have been found to be most effective. The evidence was provided much later by Scossiroli (1954) that due to mutations, possibilities of increasing the rate of evolution towards better adaptability was much more. After mutagenic treatment of seeds macromutations can be easily selected in M_2 generation, which may perhaps an excellent initial breeding material (Gustafsson and Tedin, 1954). Tedin (1954) demonstrated that after X-rays treatment, the seeds of lupine greater genetic variation may be obtained than in normal population which made selection for earliness successful without any significant decrease in yielding capacity.

Humphrey (1954) performed several experiments by using X-rays and neutrons in Soybean. Both the treatments significantly increased genetic variability for yield, plant height, maturity and seed size. The estimates of genetic variations were found on an average five times as large as those in the controls, indicating the large possibilities for selection towards yield improvement in the treated population.

Caldecott (1955) observed an inverse relationship between the water content of seeds and their sensitivity to ionizing radiations. Buzzati-Traverso (1955) stated that possibilities in rate of evolution increase more by the use of X-rays and neutrons. Experiments have been performed to study the effects of mutagenic agents on quantitative traits. Extensive work was done by Gregory (1955) on ground nut. His pioneer work was very important in radiation research as applied to plant breeding. He was able to show that samples of irradiated populations, which included only the normal types, acquired significantly greater multifactorial variability in yield than the untreated populations.

The results obtained by Gregory showed that mutations affecting a quantitative character in a crop plant can be induced by radiation and that phenotypic selection can accumulate positive mutations to produce better mutants. Goldschmidt (1955) states that the sphaerococcoid as speltoid mutants in wheat are the systematic mutations. Such mutants are much valuable for studying the phylogenetic pathways and affinities in several plants. A variation in mutagenic sensitivity of different genotypes was reported as early as 1955 by Gregory. Heslot (1959) for the first time demonstrated the mutagenic activity of ethyl methane sulphonate (EMS). Even an apparently useless mutant like the “eramosa” in *Antirrhinum* has proved to be very valuable in recombination breeding, since it enabled the development of the first non-branching type of *Antirrhinum* (Stubbe, 1959).

Blixt (1960) observed a positive correlation between seedling injury and reduction in survival in *Pisum* following treatment with different mutagens. He observed variations in mutagenic sensitivity of different genotypes of Peas. An experiment was performed by Kao *et al.*, (1960) on rice variety which shows, the genetic component of phenotypic variation for heading date and plant height was much larger in the population obtained from treated seeds than in the untreated ones. The heading date, plant height, panicle length and the genetic variability increased after treatment.

Streptomycin has been found useful for inducing mutations in extra chromosomal genetic determinants in plants (Sager 1960). Action of ionizing radiations on nucleic acids has been studied by many workers (Emmerson *et al.,* 1960; Weiss and Nakamota, 1961). The dose effect of various mutagens as measured by germination, growth rate and survival has been demonstrated by Patil and Bohra (1961) in peanut. Gaul (1961) defined two categories of mutations the macro mutations (or large mutations) and micromutations (or small mutations). Macromutations involve gross changes in phenotypes which can be recognized with certainty in a single plant. Micro mutations on the other hand can only be detected in a group and change can be measured statistically only on the basis of population. According to Gregory (1961) mutation affecting a quantitative character in a crop plant can be induced by radiation and it results for better phenotype. Matsuo and Onozawa (1961) compared the effects of X-rays, thermal neutrons and diepoxy butane applied to seeds and found that the variability for stem length and grain weight increased by these treatments, while the mean values remained unchanged. Krull and Frey (1961) estimated the effects of thermal neutron irradiation on three quantitative inherited characters *i.e.,* heading date, plant height and seed weight in oat. The Sjodin (1962) studied the dose effect of various mutagens in *Vicia faba*. He also observed that higher frequencies of mutations are obtained by the treatment of chemical mutagens. In wheat many workers have reported the use of physical and chemical mutagens for inducing variability quantitative characters (Bhatia and Swaminathan, 1962; Scossiroli and Scossiroli, 1962). In general it has been found that: (*i*) the mean value in irradiated populations is lower than the one in the control population; (*ii*) The various estimates of quantitative traits are larger in the irradiated populations than in non-irradiated ones and (*iii*) A selection data for quantitative characters is usually more effective in irradiated population.

Gaul (1964) found that recurrent radiation treatments increase the frequency of mutation in polyploids and that the mutation frequency increases with an increase in the number of radiation cycles. EMS ($CH_3SO_2OC_2H_5$) has been reported to induce chromosome breakage at the same reason in *Vicia faba i.e.* guanine and cytosine respectively (Natarajan and Upadhyaya, 1964). According to Speckman (1964) with increase the dose of EMS, decrease the seedling growth in case of pea. Swaminathan (1965) classified mutations into two major groups *viz.,* (*i*) those which can be recognized only by a study of the characters of families and (*ii*) those which can be recognized by a study of the characters in individual plants. In the later group, two sub-groups have been further identified which are based on the magnitude and types of genetic change involved, their characteristics are follows; (a) all the mutations which can be identified either the naked eye under conditions of natural growth as by the use of screening procedure such as the creation of artificial epiphytotics of diseases and the adaptation of biochemical sieves. These mutations could either be lethal or viable.

Gustafsson (1965) isolated a mutant in barley which is the example of useful macromutation. In such mutant the plant height stiffness of straw, the length breadth ratio, thickness of leaves and density of the ear effected simultaneously. Gaul (1965) pointed out two main reasons for usefulness of micromutations in plant breeding (i) they might occur much more frequently than do not affect vitality adversely as do macro mutations because minute changes pertaining to physiological behaviour are less drastic. Extensive investigations were carried out by Frey (1965) on oats. Sharma (1965) compared the effect of NMU with various other physical and chemical mutagens on pea. He observed that NMU induced the highest rate of mutations than other physical and chemical mutagens. In addition to radiation chemical mutagens are currently gaining importance in genetic studies and mutation breeding of plants. The chemicals have advantages of higher efficiency and relatively greater specificity of mutations induction their availability and easy handling. A comparison of the effectiveness of different chemical mutagens in peas also has been made by him.

The sodium azide mutagenecity in plants were discovered by Spence (1965). Santos (1965) studied the dose effect of various mutagens on germination, growth rate and survival of *Phaseolus aureus* and Zannone (1935) in *Vicia sativa*. Eiges (1965) compared the mutagenic effect of ethylamine with gamma radiation on winter wheat and indicated that ethylamine was 4 to 6 time more effective than gamma rays. Shivraj and Ramanarao (1965) treated peanut seeds with fast neutrons and observed the reduction in germination and an increase in pollen sterility, which were found to be correlated with the increase in dose. Wellensiek (1965) reported in pea a positive correlation between germination, survival and seed fertility, which decreased after treatment with X-rays, gamma rays and EMS.

Variation in the protein content of grains could be induced in the bread wheat variety N.P.-824 by treatment with X-radiation, Banerjee and Swaminathan (1966). Mugnozza (1966) treated *Triticum durum* varieties *azizioh*, *cappeli* and *russello* with both physical and chemical mutagens and observed that physical mutagens are more effective than chemical mutagens. Gaul and Aestveit (1966) has proved of the high efficiency of selection populations treated with mutagens. Sidorova (1966) concluded that the rate of spectrum of induced mutations in peas were affected by the mutagens as well as the genetic architecture of the variety. Borojevic (1966) reported use of physical and chemical mutagens for inducing variability in quantitative characters in wheat plant. Studies on induced variation for different polygenic traits in rice carried out so far have been extensively reviewed by Gustafsson and Gadd (1966). In plant like pea and barley nitroso methyl urea (NMU) has been found to be the most potent mutagen (Swaminathan, 1966a) and therefore, the chemicals belonging to this group has been named by Rapoport *et al.* (1966) as "supermutagens". Rajan and Issar (1966) treated sesame seeds with lower doses of ionizing radiation and observed stimulatory to seed germination. The increase or decrease of the mean in the treated material has explained on different ways. Based on his extensive work on subterranean clover and *Arabidopsis thaliana*, Brock (1967) proposed a general hypothesis on the behaviour of induced polygenic mutations. According to this with definite selection history, shift the treatment mean away from the control mean in the direction, opposite to the previous selection history Siddiq and Swaminathan (1968) studied the effectiveness and efficiency of EMS, NMU, NG and gamma rays on rice and observed that lower concentrations of these mutagens show higher efficiency except NG. Similarly effectiveness also followed a uniformly upward trend with decreasing concentrations. Experiment show that irradiation by thermal neutrons, X-rays and gamma rays results in a decrease in the mean value of quantitative characters in the M_1 generation as a direct effect of irradiation. The genetic behaviour of the characters plant height and number of kernels of irradiated population showed that the mean values increase sharply in M_2 when compared with M_1 (Borojevic and Borojevic, 1969). Hussein (1969) in *Arabidopsis thaliana* observed that EMS is 2-3 times more efficient in comparison to X-rays.

Sinha and Godward (1972) measured the radio-sensitivity of *macrosperma* and *microsperma* lentils. The germination and survival percentage at two leaf stage was lower in *macrosperma* than in *microsperma.* They studied the effect of gamma rays on habit, branching, number of flowers, pods and seeds. The reduction in all these attributes was more in *microsperma* than in *macrosperma* at the low dose level. The roots appeared to be more sensitive towards mutagenic action than the shoot. Abo-Hegasi (1973) used seeds of five Leguminous plants *viz., Vicia faba*, *Lens culinaris, Trigonella foenum-graecum, Cicer arietinum* and *Lupinus ternis* to study the extent of variation generated in the M_1 generation following gamma irradiation. He observed that co-efficient of variation was increased for number of days to flowering, number of pods, number of branches, plant height and seed yield per plant.

The data collected by Mandal (1974) on the quantitative characters in gram showed a significantly higher variability in the treated material in respect of height, length, breadth of the leaf, number of pods per plant, number of seeds per pod and 100 grains weight. An analysis of co-efficient of variation revealed that for all the characters studied the variability in M_2 population was larger than in control, irrespective to treatment employed. Raghuvanshi and Singh (1974), studied the mutagenic effect of colchicine and gamma rays in *Trigonella foenum-graecum* L. They isolated male sterile plant having bushy habit. Its gynoecium was found to be normal. In colchicine treated plants, increased branch number (bushy habit) was recorded at 0.03% concentration level, which again decreased with increasing treatment dosage. Similarly, Rao (1974) reported in red gram that the overall variance of the seed number per pod, pod number per plant, and single plant yield was higher in the treated progeny as compared to control. It was possible to generate significant amount of variability for number of characters in Pigeon pea. The characters studied were number of branches per plant, raceme length, number of pods per raceme, seeds per pod, 1000 seed weight and single plant yield. The variability was studied at various levels including overall intra-family and inter-family variance. The pattern of induced variability showed that considerable scope existed for making selection within and between the families.

Khalatkar *et al*. (1975) determined modification of mutagenic efficiency of EMS with ethidium bromide, iodoacetamide and sodium fluoride in barley. He also compared the effect of EMS and few other chemicals on germination of barley. In ground nut (*Arachis hypogaea*), Sharma and Kant (1975) observed significant increase in overall variance and interfamily variance for number of pods, 100 seed weight and seed yield per plant. The magnitude of heritable variation and the estimate of genetic advance varied according to the mutagen treatment. The characters such as weight of pods, seed yield and number of secondary branches showed a higher response to mutagenic treatment, indicating that remarkable opportunities exist for improvement in their characters.

Hussein and Disouki (1976) studied the effects of EMS and gamma rays on *Phaseolus vulgaris* L. They reported a decrease in germination, survival and fertility in M_1 generations with the increase in the dose of gamma rays and EMS. He also observed a notable difference in mutagenic sensitivity of different varieties of *Phaseolus vulgaris* L. The high tolerance to golden mosaic virus disease was induced by EMS seed treatment of dry bean (Tulmann Neto *et al*., 1977). Verma *et al*. (1977) studied the effect of gamma rays and EMS on the two varieties of *Brassica juncea* and reported that both the varieties are equally sensitive. Abidi *et al*. (1978) while studying the effect of acute gamma irradiation on seed germination of *Linum usitatissimum* variety *neelum* observed that gamma rays effectively promote the germination process. Chaghtai *et al*. (1978b & c) demonstrated that presoaked seeds are more vulnerable to the effect of radiations than the dry ones in *Lens esculenta* and *Phaseolus mungo*. Chaghtai *et al*. (1978a) statistically compared the effects of EMS and MMS on the leaf lamina of sunflower. Variations of leaf lamina of sunflower under the influence of EMS were also studied by Chaghtai and Hasan (1978b). Sasakuma *et al*. (1978) worked out the EMS sensitivity to induce mutation in wheat.

Ranghuvanshi and Singh (1981) observed high heritability estimates and genetic advance for double pod trait implying that selection will be effective for improvement in this trait. Badar and Elkington (1982) studied antimitotic and chromotoxic effect of isoproturon on plant chromosomes. Bahl and Gupta (1982) described the mutant characters and their inheritance in mung bean when treated with EMS and gamma rays and then reported that *Variegated*, *Multifoliata*, *Xantha*, *Chlorina*, *Albina*, *Unifoliata* were each controlled by a recessive gene. Subtoxic level of 2,4-D promotes greater localization of DNA during transition from vegetative to reproductive phase in *Trigonella foenum-graecum* L. Quicker leaf and bud primordial differentiation during the onset of flowering in the treated plants are also reported (Hariharan and Unnikrishan, 1982).

Mani (1982) studied the effect of IAA with Maleic hydrazide and colchicine on root tips, mitotic division and induction of chromosomal abnormality in *Allium cepa* root tips treated with 25, 50 and 75 ppm concentrations for 4, 6 and 12 hour duration. Inhibition of mitosis was brought about under all treatments except IAA, which at the inhibition of mitosis was most effective and MH was least effective. Jain and Agarwal (1987) treated the seeds of *Trigonella corniculata* and *Trigonella foenum-graecum* L. with different concentration of EMS, MMS and SA (NaN_3) separately to study the effect on the level of ascorbic acid. A decrease in percentage of germination was observed in treated sample but an increase in ascorbic acid content was recorded at low concentration followed by a continuous decrease in its level with higher concentration of used mutagens in both the plant species. Mutagen treatment induced seedling injury, lethality, pollen sterility and morphological abnormalities besides delayed leaf initiation in M_1 generation (Laxmi and Datta, 1987). According to Pathak and Patel (1988) the germination decreased after gamma irradiation in rice. Mishra and Raghuvanshi (1989), studied cytogenetic effect of gamma irradiated stored seed of *Trigonella foenum-graecum* L. Gamma irradiation is one of the most important physical mutagens which is used for cytogenetic effects. The cytogenetic changes occurring due to the storages of *Trigonella foenum-graecum* L. seeds (2n and 4n) after gamma irradiation were investigated. This treatment accelerated the mitosis and decreased/aberration in diploid (2n) as well as on tetraploid (4n). Chromosomal changes produced fragments and stay in metaphase cell, laggards, fragments and bridges in anaphase. Appearance of higher frequency of aberration per cell in tetraploid indicated that tetraploids were more sensitive to gamma irradiation than the diploids. This study also reveals that the storage of seeds after gamma irradiation may lead to genetic repairing.

Devi and Reddy (1990) studied sensitivity to chemical mutagens like, ethyl methane sulphonate, diethyl sulphonate and ethylene imine in *Trigonella foenum-graecum* L. They noticed meiotic abnormalities like bridges, fragments, laggards, pre-cocious movements and orientation of chromosomes. Swamy *et al*. (1990) studied effect of two hydrazones namely *bahna* (benzoic acid hydrazone of 2-aminonicotinaldehyde) and *scana* (semicarbazone of 2 aminonicatinaldehyde) on mitosis in root tips of *Trigonella foenum-graecum* L. The mitotic index fell down with gradually increasing concentration of hydrazones. *Bahana* is more potent chemical in inducing chromosomal aberrations than *scana*.

The effects of gamma-irradiated sludge on the growth and yield of methi (*Trigonella foenum-graecum* L.) in plot cultures have been studied. Gamma irradiated studge was found to inhibit the shoot length after 45 and 90 days of plant growth compared to plants grown in soil containing unirradiated sludge. The significant effect of gamma irradiated sludge was observed on the biochemical growth parameters *i.e.*, increase in total protein content, total soluble sugars and starch contents after 45 days of growth. Above biochemical parameters studied even after 90 days of plant growth but it shows inhibitory to protein and starch content of plant in latter stages of plant growth. This shows beneficial effect of recycling of irradiated sludge for agricultural applications. (Pandya *et al*., 1991). Patel *et al*. (1991) studied the effect of different levels of M_2, Phosphrus and Potash on yield and yield attributes grown on loamy sand soil. Siddiqui (1991) studied induced variability in pusa purple long (PPL) of egg plant (*Solanum melongena*) treated with hydrazide and maleic hydrazide at 0.2%, 0.6%, 0.8% concentration. The phenotypic co-efficient of variability was highest in treated population of both mutagens. On this basis of phenotypic variability, it is expected that the further improvement of pusa purple long is possible through induced chemical mutagenesis.

Mutation frequency, effectiveness and efficiency of gamma rays, EMS and its synergistic effect of their combination treatment was assessed in black gram variety T-9, gamma rays more effective than EMS. However EMS was 2-2.5 times more efficient than gamma rays (Gautam *et al*., 1992). An investigation was carried out by Mishra (1992) to assess the effect of various doses of gamma rays on genetic improvement of yield and its attributes *viz*., seeds per Plant, 100 seed weight and harvest index in M_2 and M_3 generations of gram (*Cicer arietinum*). The analysis made in both the generations revealed that 20 Kr gamma rays were most effective to increase the mean performance of seed number and yield per plant. 30 Kr and 40 Kr were more effective in increasing the range of genetic variability in both the M_2 and M_3 generations.

Reddy *et al*. (1992) studied the effect of gamma irradiation, EMS and SA on meiotic division in lentil. The seeds were treated with various doses and concentration of gamma rays. In the case of EMS and sodium azide, the aberration recorded in M_1 generation were quadrivalents, trivalents ring and rod bivalents, unoriented chromosomes, bridges with fragments, micronuclei, pollen sterility and number of seeds per plant, meiotic abnormalities increased with dose, duration and concentration of mutagen combined treatment exhibited higher abnormalities. A gradual decrease in percentage of germination, seedling growth, internodal length, number of nodes, flowers and pods per plant, number of seeds per pod and weight of seeds in M_1 and M_2 generations were observed when the seeds of *Trigonella foenum-graecum* were treated with different concentration of EMS, MMS (Jain and Agarwal, 1993). A number of morphologically different plants such as abnormal leaf type, dwarf type, early flowering, double flower, double pods have also been isolated.

Singh and Sharma (1993) isolated a few pentafoliate and tetrafoliate mutants from the gamma rays and EMS treated mungbean. TMD-1 variety of bean (*Phaseolus vulgaris* L.) is resistant to golden mosaic virus disease obtained through cross breeding programme using an induced mutant (Tulmann Neto *et al.*, 1993). Mahana *et al*. (1994) studied the effect of alkylating agents and gamma rays on the diosgenin production in the induced mutants of *Trigonella corniculata*. Significant increase in the level of diosgenin was observed in tall, heterophyllous leaves and an irregular mutant with significant decrease in its level was recorded in *Virgin xantheceens* mutants. Little variation in diosgenin content was recorded in dwarf excessively branched, unifoliate, high nodulating mutants, so it is possible that there is possibility to increase diosgenin level in *Trigonella* plants through induced mutagenesis.

According to Cerdon *et al*. (1995) Tetcyclacis, a corbomanodiazetine plant growth retardant, used at 10 mg L^{-1} (36 m M), caused greater growth inhibition in the shoots of fenugreek seedlings (60%) than in the shoots (30%), compared with control. The greater retardation was reversed by a supplement of gibberellins (200 mg L^{-1}). The tetcyclacis resulted of modification in sterol profile, leading to an accumulation of 14a- methyl sterols. It also caused a significant increase in the cholesterol content of roots: 38.1% of total sterols against 3.7% in the control roots. A decrease in sapogenin content of treated roots was noticed. According to Singal *et al.* (1995) with increasing the Cd^{+2} concentrations there was a significant decrease in root, shoot length and fresh mass. Various phosphorous fractions of shoot decreased with increasing Cd^{+2} concentration except lipid P and nucleic acid P which increased at 65 and 95 days after sowing and protein P only increased at vegetative stage .

Quin *et al*. (1995) have also observed an inhibitory effect on root development among regenerated *Brassica oleraceae* L. plants after gamma irradiation. Siddiqui and Khan (1995 and 1996) reported mutation genetic studies in mung bean-I and variability components, genetic parameter, frequency and spectrum of morphological mutants in mung bean-II. According to Alcantara *et al*. (1996) the concentrations and duration of seed exposure to EMS could be increased to induce even greater number of mutants in *Capsicum annuum.* Very high frequency of point mutations was observed for other barley genotypes after combined treatment with NaN_3 and MNH. It is proven that the combined treatment of NaN_3 and MNH yielded a wide spectrum of gene mutations in many barley genotypes, including dwarf and semi-dwarf characters, changes in root system development and structure. Also mutants with an increased level of tolerance to Al^{+2} toxicity were selected Nawrat *et al*. (1997).

Gautam *et al.* (1998) in rajmash (*Phaseolus vulgaris* L.) studied the effectiveness and efficiency. The combined treatments of gamma rays and SA in *Oryza sativa* L. resulted in larger in culm length (CL) than for the treatments with the gamma rays alone. Combined treatments with gamma rays and SA did not increase the variance and tiller number (TN) when compared with the corresponding single treatment (Montalvan *et al*., 1998). Sareen *et al*. (1999) reported the promontory effect of gamma rays on germination percentage between 60 Kr to 120 Kr. Seedling height decreased with increasing doses of gamma rays. Venkatachalam *et al*. (1999) studied on twelve new groundnut (*Arachis hypogea* L.) mutated germplasm. Two mutant lines of high yield and oil content, one mutant of disease and on of drought resistance and six mutants for pod, kernel and improvement of other characters were identified. According to them well developed uniform and dry seeds were irradiated with gamma rays from a “co-source. The seeds were treated with various doses of ethyl methane sulphonate (EMS) and (NaN_3) sodium azide.

Singh and Rathore (2000) reported the decreasing trend of the dry weight and pollen fertility with increasing doses of mutagen treatment in isabgol. Four chemical mutagens, EMS, ethidium bromide (EBr), ethyl nitroso urea (ENU) and streptomycin were used to induce mutations in *Brassica juncea*. EMS and EBr were found to be highly efficient and yielded mutants for all the traits examined (Bhatt *et al*., 2001). Induced mutation in *Trigonella foenum-graecum* L. was studied by Choudhary and Singh (2001). Mutagenic effectiveness and efficiency of gamma rays, ethyl methane sulphonate and their combination in mungbean (*Vigna radiata* L.) Wilezek (Singh and Singh, 2001). According to Cantor *et al*. (2002) the effect of gamma radiation and magnetic field exposure showed the variability in the case of *Gladiolus*. The result showed that when we increasing level of Phosphorus up to 40 Kg P_2O_5/ha and Potassium up to 45 kg/ha significantly increased all the growth characters (plant height, dry matter per meter row length, branches per plant etc.) yield attributes, yield, net return and B : C ratio/hectare as compared to other P and K levels (Nehra *et al*., 2002).

Gupta and Kumar (2003), when seeds of *Trigonella foenum-graecum* L. were pretreated for 24 hours in variable concentration of different growth regulators (IAA, IBA, NAA and GA_3) and were allowed to germinate. Maximum percentage of seed germination was obtained at 1 and 10 ppm, 0.5 and 1 ppm IBA, 0.5 ppm NAA and 10 ppm GA_3. Lower doses of IAA (0.5 and 1 ppm) promoted shooting. Higher doses of GA_3 *i.e.*, 50 and 100 ppm significantly improved shooting. IAA induced maximum rooting while GA_3 induced maximum shooting. The plants treated with growth regulators showed improved growth and productivity under field conditions also.

A study on genetic variability and selection criteria in F_3 generation in fenugreek was made by Mahey *et al*. (2003). A field experiment was conducted on loamy sand soil to study the effect of iron, molybdenum and *Rhizobium* inoculation of fenugreek (*Trigonella foenum-graecum* L.). Application of iron at 0.5 kg/ha and seed inoculation with *Rhizobium* significantly increased plant height, dry matter accumulation/metre row length branches/plant, number and dry weight of root nodules/plant, pods/plant, seed, straw and biological yields control. Seed pod and test weight were also significantly higher with *Rhizobium* inoculation over no inoculation (Kumawat *et al*., 2003).

Hewawasam *et al*. (2004) observed that by increasing dose of both mutagens like, gamma rays and colchicines the mean shoot length reduces and by increasing the dose of colchicines the average number of shoots increases the treatment of 3 Kr gamma radiation produced a solid mutant with altered leaf shape and flower colour of *Crossandra infundibuliformis* var. dania. On the basis of the conducted investigations, Diana and Crino (2005) concludes that the treatment of leaf petioles explants by ethyl methane sulfonate (EMS) and N-nitrose-N'-ethyl urea (EMU) influenced callus growth of common bean. Treatment of explants with such chemical mutagens for 60 minute can be optimal in case of investigations of the regeneration capacity in common bean (*Phaseolus vulgaris* L.). EMU evidenced an inhibition effect stronger than EMS. The experiment observed range of different morphometric parameters of the M_1 and M_2 mutant plants generated due to the different doses of EMS (Acharya *et al*., 2005).

In India, Swaminathan and his team at IARI, New Delhi initiated a major programme on mutagenesis in crop plants. These studies were broadly aimed at understanding the process of mutation testing the efficacy of various mutagens, identifying optimum dose and the best method of treatment for different crop species ; isolation of mutants of basic and applied value; elucidating the biological effects of radiation treated media, seeds and vegetative propagules on the organisms consuming them (Chopra, 2005). Tariq *et al*. (2005) studied radiation induced pod and seed mutants and MMS induced closed flower mutants in broad bean (*Vicia faba* L.). A mutagenesis programme was carried out using three chemical mutagens *viz*., EMS, MMS and SA on two variants of cowpea (*Vigna unguiculata* L.). The M_1 generation was raised only from higher doses of mutagens which adversely affected their survival. The MMS treatment was found to be most effective and efficient. RC 19 followed by SA and EMS while on RC 101, SA was most effective indicating differential response of the genotypes (Singh *et al*., 2006). Kumar and Gupta (2007) concludes that when the seeds of *Nigella sativa* were treated with 50, 100, 150, 200, 300Gy doses of gamma rays, a hexapetalous mutants was observed at 100Gy dose in M_1 generation found to have increased seed number and seed weight.

Chlorophyll mutants

Gregory (1956 and 1957) worked on groundnut which is very important in radiation research as applied in plant breeding. During his work he observed that irradiated populations showed significantly greater multi-factorial variability in yield than the untreated populations. The higher frequencies of chlorophyll and other viable mutations are obtained in treatments with chemical mutagens than radiations, Blixt *et al*. (1958). Oka *et al*. (1958) found that after X-irradiation in rice seeds, the genetic component of phenotypic variation for heading date and plant height was much larger in the population obtained from treated seeds than in the untreated ones. Among the chemical mutagens, alkylating agents especially EMS was demonstrated to be the most potent. An interesting observation was that genes near the centromere were more prone to mutagenic treatment than those located farther away. Chlorophyll mutants were frequent in EMS treatment but were rare in treatments with physical mutagens (Pal *et al.,* 1958).

Chlorophyll mutation has also been induced by following exposure to chronic gamma radiation in rice (Basu, 1962). A wide range of X-rays induced mutant in black gram, ranging from completely sterile to fertile have been reported by Jana (1962). Shivraj *et al.* (1962) treated peanut seeds with fast neutrons and observed the reduction in germination, increase pollen sterility. EMS induces a high frequency of chlorophyll and morphological mutations in emmer wheat (*Triticum dicoccum* var. Khapli; 2n = 28) (Swaminathan *et al*., 1962). According to Rapoport (1963) the nitroso compounds have been found to be most effective than all other chemical mutagens. The seedling growth declined in pea with the increase in the dose of gamma rays. It has been noticed that temperature, water content, oxygen tension, protective substance and in the seed and the type of ionizing radiation effect the seedling growth (Blixt *et al*., 1963). According to Bhatia and Swaminathan (1963) in wheat, among the various physical mutagens examined, thermal neutrons were found to be the most effective followed by ^{35}S, ^{32}P and X-rays. Abrans and Frey (1964) compared the effects of thermal neutrons with EMS and ^{32}P and found that thermal neutrons were a little more effective than other mutagens in inducing genetic variability for heading date, plant height and seed weight.

Chopra and Swaminathan (1967) studied the comparative effect of EMS, hydroxylamine and their combination treatments on emmer wheat and observed that chlorophyll and viable mutation frequency in M_2 was higher under EMS treatment. Blixt and Monaberg (1967) compared the effectiveness of different chemical mutagens in peas. Savin *et al*. (1968) compared the effect of two monofunctional alkylating agents EMS and NMU on germination of barley seeds and observed that EMS (0.3%, 2 and 4 hours) and NMU (0.3%, 2 hours) have a similar trend with regard to influence of presoaking on seed sensitivity. He also observed that in barley NMU is superior to EMS in inducing chlorophyll mutations. Multiple mutations also occurred in the NMU treated plants. Reduction in plant height in many tropical ornamental plants after exposure to different mutagenic agents has also been reported by Heslot (1968). Monti (1968) obtained very high frequency of chlorophyll and viable mutations with the treatment of DES (diethyl sulphate) than X-rays. He also reported that in peas the effectiveness of DES was 3 to 4 times higher than that of X-rays, estimated either on the basis of chlorophyll or morphological mutations or both.

Kawai (1969) used many chemicals and radiations on different varieties of rice and observed that EMS induced chlorophyll mutations at a rate about three times as high as that induced by radiations. Other effective mutagens in these experiments were N-nitroso-N-methyl Urea and N-nitroso-N-methyl urethane. Grinikh (1970) reported that in *Crepis capillaris* effectiveness of EMS is altered with a slight change in copper ion concentration and pH. He also correlated the temperature and effectiveness of EMS in *Crepis capillaris*. Jacob (1970) studied the comparative mutagenic effects of alkylating agents and gamma rays, and observed that EMS induced highest chlorophyll mutation frequency as comparison to MMS, MNG, BMS and gamma rays. The following order of efficiency of various mutagens recorded was EMS > MNG > MMS > gamma rays > BMS in the case of *Arabidopsis thaliana*. Marki and Bianu (1970) reported that EMS and NMU were more effective than gamma rays in inducing chlorophyll mutations and they also produce a different spectrum of mutations.

Nerkar (1970) in *Lathyrus sativus* found that NMU and EMS were more effective in inducing chlorophyll mutations than gamma rays. But, while considering the viable mutations alone, NMU was found to be more potent mutagen. He also observed that varieties belonging to the different sub-species differ in radio- sensitivity in *Lathyrus sativus*. He observed that LD-50 dose of gamma rays on the basis of survival was 10 Kr for the variety *tabrin*, 10 to 15 Kr for the varieties T-2-12, Rewa-2, L-C-76 and 15 Kr to 20 Kr for the variety Rewa-1. The chemical mutagens EMS and NMU used in concentrations of 0.1 to 0.5% and 0.001% to 0.01% respectively induced greater pollen sterility than the corresponding doses of gamma rays in the range of 5 to 50 Kr. Redei and Li (1970) confined that in *Arabidopsis thaliana* EMS was decidedly more effective than X-rays. Bari (1971) found significant difference in germination of *Lilium* seeds given acute gamma irradiation. Delaflora *et al*. (1971) worked out the mutation frequencies induced by EMS during different phases of seed germination of common bean (*Phaseolus vulgari*s). Nayar (1971) observed X-rays sensitivity on *Sesamum* seeds and found reduction in germination, survival and flowering etc.

Ramulu (1971) investigated the comparative effect of physical and chemical mutagens and concluded that EMS was the potent mutagen. Solodyuk (1971) used EMS and NMU in '*kiev early*' variety of *Lupinus albus*. He found that spectrum and frequency of mutant characters were more dependent on the nature of the mutagen. Shree Ramulu (1971, 1972) compared the effect of nitrosomethyl urea (NMU) and nitroso guanidine (NG) in *Sorghum* and noticed that NMU caused greater damage in germination, survival, seeding growth and fertility in M_1 generation than nitroguanidine. He also observed that NMU was more effective and efficient as it induced higher frequency and wider spectrum of chlorophyll mutations than NG. Swaminathan *et al*., (1971) observed that frequency and spectrum, induced in different rice varieties by physical and chemical mutagens. Also he studied the efficiency of physical and chemical mutagens, on dehusked seeds of rice under different pressure conditions. Prasad (1972) compared the mutagenic effectiveness and efficiency of gamma rays, EMS, NMU and NG using *Triticum durum* variety NP 404 as the test material. He found EMS to be the most effective and reasonably efficient mutagen and stated that it could be a much useful mutagen at low concentrations. He concludes that a higher frequency of chlorophyll mutations was induced by NMU followed by gamma rays and EMS.

Gaponenko *et al*., (1973) studied the frequency and spectrum of chlorophyll mutations in barley after treatment with NMU. Siddiq (1973) studied the effect of physical and chemical mutagens in various rice varieties and observed that chemical mutagens are more potent. The vegetative growth decreased with an increase in the dose of chloro choline chloride or cycocel (CCC) as indicated by the final plant height and air dry weight of cotton stalks at the time of harvest (Singh *et al.*, 1973). Gichner and Veleminsky (1974) treated barley seeds with caffeine and EMS and demonstrated that caffeine post treatment potentiates the effect of EMS during M_1 seed germination and EMS potency was found dependent on caffeine concentration. Hussein *et al.* (1974) studied the frequency and spectrum of M_2 chlorophyll mutations in *Pisum sativum* after treatment with EMS, gamma rays, Iodoacetamide and sodium fluoride in barley. He also compared the effect of EMS and few other chemical on germination of barley.

Sahai (1974) studied the mutagenic sensitivity of the two species *Phaseolus aureus* and *Phaseolus mungo*. It was observed that LD-50 dose for *Phaseolus aureus* was higher than for *Phaseolus mungo*. In case of *Phaseolus mungo* the early maturing varieties were found to be more sensitive than the late maturing ones. Thus within a spices, differential mutagenic sensitivity was established. He concluded that NMU is the most effective mutagen as judged by induction of chlorophyll mutations in few varieties of green gram. It was followed by EMS and gamma rays. However, in the case of viable mutations, EMS turned out to be more effective than NMU and gamma rays.

Swietlinska and Zuk (1974) found that maleic hydrazide (MH) deoxybutane, N-ethyl-N nitroso urea (NEU) and methyl methane sulphonate (MMS) were more sensitive to caffeine. Induction of aberrations by MMS and ENU were highly potentiated by caffeine post treatment in *Vicia faba*. They also found that the yield of aberrations induced by X-rays in rye seeds was significantly increased by caffeine. The potentiating effect of caffeine as the chromosome damage induced by ionizing radiation was less pronounced. Desai and Bhatia (1975) used EMS and NMU of equimolar concentrations on durum wheat to study the mutation rate. They observed a higher chlorophyll and viable mutation frequency with NMU treatment. The efficiency of NMU was higher than that of NEU at equimolar concentrations on durum wheat. Liwerant and Pereira (1976) studied the comparative mutagenic effect of EMS, NMU and few radiations on *Distostelium discoideum* and found that chemicals are more effective to induce the chlorophyll and other mutations. He also studied that mutagenic effectiveness of EMS, NMG, UV-radiation and caffeine in *Distostelium discoideum* and concluded that the chemical mutagens are more effective than UV-radiation.

In Niger (*Guizotia abussinea*), Nayakar (1976) reported genetic variability for six quantitative characters and observed lowest genetic variability, low heritability estimates and a high expected genetic advancement. Chemical mutagen ethidium bromide (EBr) has been reported to induce cytoplasmic male sterility in pearl millet (Burton and Hanna, 1976). Kaul and Bhan (1977) studied the mutagenic effectiveness and efficiency of EMS, DES and gamma rays in rice. They observed that EMS is more potent than DES and gamma rays. Meono (1977) studied the chlorophyll mutation frequency induced by gamma rays in *Phaseolus vulgaris*. Race (1977) concluded that in rice, EMS was more effective and efficient in comparison to gamma rays. Khan (1988) studied the effect of gamma rays and EMS in single and combination treatments on seeds germination, seeding growth, survival, pollen and seeds fertility, recovery index (RI) in M_1 and frequency and spectrum of chlorophyll mutations in M_2. The percentage of germination, growth of seedling and survival decreased with an increase in the dose of mutagens used. Combination treatments caused more drastic effects than the single treatment. Priyadarshan *et al*. (1989) have required tissue-culture work on fenugreek. Bansal *et al*. (1990) reported most mutagenic effectiveness of gamma rays, EMS and other combined treatments in rice. Deore and Bharud (1990) observed that growth substances *viz*. Ascorbic acid, IAA, GA_3 and urea improved the yield and physico-chemical characteristics *i.e.* ascorbic acid content, chlorophyll content and total acid content of leafy vegetable fenugreek significantly over the control. But GA_3 was superior among ascobic acid, IAA and urea.

Kaushik and Dashora (2001) studied the action of ionizing radiations on nucleic acids. According to Kumawat and Majumdar (2001) an increasing levels of compaction increased the growth attributes *viz*. (plant height, branches per plant, chlorophyll content, number of effective and total nodules per plant), yield attributed (number of pods per plant, number of seeds per pod and test weight), yield (seed and straw yield). Sulphur also shows the almost same attributes *viz.,* (plant height, branches per plant, number of effective and total nodules per plant, attributes (number of pods per plant, number of seeds per pod and test weight), yield (seed and straw yield). Interactive effect of compaction and sulphur significantly increased effective and total nodules per plant yield attributes (number of pods per plant and number of seeds per pod) yield (seed and straw yield).

According to Cheema and Atta (2003) in basmati rice the increase in radiation doses of gamma rays the decrease in germination, seeding height, root length and emergence under field conditions was observed in M_1 generation. The frequency of chlorophyll mutations in M_2 generation increased with the increase the radiation doses upto 250 Gly which sharply decreased thereafter. Avtar *et al*. (2003) gather information on nature and magnitude of gene effects for biological and seed yield in fenugreek. The frequency of morphological and chlorophyll mutations increased with increasing doses of gamma rays in Isabgol (*Plantago ovata* Forsk). In variety RI-89, the gamma rays treatment between 45 Kr to 90 Kr were found most appropriate (Jain *et al.,* 2005). The chemical induces genetic sterility in rice without changes in vigour (Mensah *et al*., 2005).

According to Mensah *et al*. (2007) the dose related effects of the mutagenic treatments (sodium azide and colchicine) on quantitative traits resulting in reductions in traits such as germination, survival percentage, plants height and number of fruit/plant, but increases in leaf area, maturity time and fruit size. Colchicine treatment produced shortened internodes deformed leaves, and chlorophyll mutants. Low doses of both mutagens produced early maturing variants and robust seed/high yield and can be imposed to obtain beneficial mutants in sesame.

The radiomimetic agents like, EMS (Ethyl Methane Sulphonate), MMS (Methyl Methane Sulphonate) and MES (Methyl Ethane Sulphonate) induce plant height and number of pods per plant in two varieties of *Trigonella foenum graecum* L. (Vasu and Hasan, 2009).

An experiment was conducted during winter seasons on morphological characters of Lentil (*Lens culinaris Medic.*) enoculated with PSB. PSB (10gm, 20gm and 30gm) in 2Kg of soil and one control. Number of Flowers per plant is more in both variety JL-3 and NDL-92 inoculated with PSB as compared to control. Number of Pods per plant are more in both varieties JL-3 and NDL-92 inoculated as compared to control. Dry weight/plant is more in inoculated JL-3 and NDL-92 plants as compared to control (Kumari, *et al.,* 2009).

Maximum increase in the growth parameter was with combined treatment of Azotobacter + PSB inoculated crop and then the crop treated with Azotobacter showed somewhat less growth parameters and the least growth parameters in these three inoculations with PSB treatment **(**Kumari, *et al.,* 2010).

In some experiments auxin 2, 4-D, NAA and cytokinin BAP, Kinetin were used for optimization of maximum callus induction. (Vasu, *et al.,* 2014). Hybridization between genotypes from cluster III and cluster IV for these characters can produce better segregants in segregating populations (Kole and Goswami, 2015). This is concluded that SSR markers could efficiently clarify the existent genetic variability in olive, and the identified genetic variability is somewhat in coincidence with the geographical distribution of olive genotypes (Ali Bahmani *et al.,* 2016). Zinc foliar (0.68 kg/ ha) was applied in an experiment equipped with foliar application. The agronomic trait of grain yield was measured per plant. Moreover, the two content traits of zinc and iron in whole grains were assessed using DTZ and PPB methods and scans obtained by Photoshop software. The analysis results of variance showed presence of high genetic diversity in terms of the traits considered in this study **(**Nasibeh *et al.,* 2016). Cluster analysis of the genotypes indicate that hybridization between genotypes from cluster III and cluster IV for these characters can produce better segregants in segregating populations (Tariyal, *et al.,* 2017). High heritability coupled with high genetic advance was observed for grain yield per plot and straw yield per plot indicating the importance of additive gene action in governing the inheritance of these traits (Jyothsna, *et al.,* 2016).

Path analysis studies revealed that number of pods per plant showed true relationship by establishing positive association and direct effect on seed yield both at genotypic and phenotypic levels and plant height and length of pod at phenotypic level and number of seeds per pod at genotypic level (Jyothsna, *et al.,* 2016).

A study was carried out in this background and two male specific SSR markers for *C. thwaitesii* were identified. The diagnostic potential of microsatellite markers can be exploited to identify the sex of the plant at early seedling stage (Binoy Kurian and K. K. Sabu 2017).

CONCLUSIONS

The effectiveness of radiomimetic agents increased and efficiency decreased with the increasing dose rate: Nevertheless highest mutation rates were obtained to higher doses under MMS treatment. The researchers in various studies found that the experimental material was analyzed for induced polygenic variability it was found that in the treated population with radiomimetic agents the mean value of some of the characters decreased in M_2 but subsequently increased in M_3 generation. The dispersion in the average values of these characters in M_2 is probably the outcome of residual damaging effect of radiomimetic agents EMS, MMS and MES of treatments and improvement in M_3 is due to the recovery from such damage.

The spectrum and relative proportion of different chlorophyll mutations were more or less similar in most of the crops. Different morphological mutations were also recorded in most of the studies. The mutation affected almost all parts of the plant. The spectrum of morphological mutations was narrow in few varieties and broader in others. The mutation spectrum has been found to be dependent upon the nature of mutagen used. There was no apparent relationship between the dose of mutagen and the extent of variability induced. It has been inferred that selection should be made preferably from M_3 generations, where the genetic variability is clearly manifested and some genetic stability is reached.

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