Chapter-2
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Mutations have served as a vehicle of progress in evolution as well as improvement of living organisms in terms of their economic utility (breeding). Variability at the level of gene (DNA) can be created through mutations. Grossly speaking, mutations are grouped into two major categories on the basis of their phenotypic manifestation:

(i) Macromutations - with large change in the characters which can be detected even without instrumental help at the level of individual organism (plant), and

(ii) Micromutations - with minor changes in the properties which are practically unidentifiable in an individual plant but can be measured at the level of population using various statistical parameters, such as, character mean, variance, etc.

Macromutations, whether resulting from single-gene changes or chromosomal aberrations, behave as monogenic traits and follow the Mendelian pattern of inheritance. On the other hand, micromutations are governed by the principles of quantitative genetics. Even since the early part of the history of induced mutagenesis, it has been a well known fact that even “monogenic” macromutations are invariably associated with multiple pleiotropic effects. Some of which (e.g. chlorophyll deficiency, sterility, reduced productivity, etc.) make them unsuitable for plant breeding. In contrast, micromutations, being subtle
changes in a large number of loci associated with determination of plant morphology or physiology, have negligible "side effect". It has been generally believed that such mutations for any economic trait could be accumulated in a single genotype to great advantage.

In spite of these expectations, micromutations for polygenic traits have not been of much consequence in plant breeding, whereas hundreds of plant varieties have been evolved using macromutations directly or indirectly. Compiled information on this aspect can be found in the issues of the Mutation Breeding Newsletter published by the International Atomic Energy Agency (IAEA), Vienna.

It is well known that a crop plant can be improved in productivity, resistance to pest and adaptation to environment when genetic variability for the specific trait is available in the considered population or species. The process of breeding crop plants has been successful for a long time, because genetic variation already present in the population had been used, and subsequently further genetic variation was made available by crossing plants from different populations, varieties, species and genera. In some cases, however, for instance in bread wheat, the progress obtained for productivity has exploited the variability present in nature to such large extent that only further progress from the classical methods of breeding become more and more difficult (Natarajan et al., 1985).
The possibility offered by mutagenic agents to induce new genetic variation is, therefore, of extreme interest. It might in many cases be the only answer to problems posed upon the practical breeder. A mutation event is indeed very important even when it has a small effect for a specific morphological or physiological character, because it changes the balance established by natural selection in co-adapted blocks of genes and it, therefore, offers new situations for natural or artificial selections.

Exposure of a biological material to a mutagen in order to induce mutation is known as mutagenesis. When mutations are induced for crop improvement, the entire operation of induction and isolation of mutants is termed as mutation breeding. Various considerations like part of plant to be treated, mutagens, dose of mutagens, methods of treatment, modifying factors, and methods of pre-and post-treatments constitute what is precisely known as mutagenesis technique.

First observations about artificial induction of genetic changes date back to the beginning of the 20th century (Gager, 1908), but proper proof of Mendelian inheritance of such induce changes came only in the late twenties by Muller, Stadler and others using X-rays as mutagens (Muller, 1927; Stadler, 1928a, b). Although Muller, being an entomologist, assumed that induced mutations could play an important role in further genetic improvement of plants, Stadler as a plant breeder became rather special about such prospects when he noticed so many useless and even deleterious mutations in maize and barley. Stadler’s specticism has influenced almost two generations of plant breeders, especially in North
America, and has led to a widely spread preconceived notion that mutation induction will be of high interest to geneticists, but is a rather wasteful undertaking for plant breeders. Stadler’s view, primarily, was based upon this experiments with maize, where a lot of genetic diversity exists (as in most cross-pollinated crop), from which improved varieties could still easily be developed simply through selection or a combination of cross breeding and selection.

Among the first researchers who used mutagenesis strictly for plant breeding were Freisleben and Lein in Halle (Germany). They succeeded in obtaining mildew resistance in barley (Freisleben and Lein, 1942) and developed a practical mutation breeding procedure (Freisleben and Lein, 1943a, b), but due to World War II this work was not followed up properly (Hoffmann, 1959). In the meantime, primarily in Sweden, plant geneticist such as Nilsson Ehle, Gustafsson, Hagberg, Gelin and Nybon continued to experiment mainly with X-rays and carried out rather systematic studies as to optimal doses, treatment conditions, mutation frequency and mutation spectra. They also compared X-ray effects with those of certain chemicals which became known as mutagens, such as (EI) ethylenimine (Micke et al., 1980). Although most of this work was of a fundamental nature, there were by-products which turned out to be of interest to breeder-easily recognizable mutants of barley, wheat, oats with early or later heading, short straw or different spike architecture but also mutants of pea, soybean, flax, mustard and rape (Gustafsson, 1947).
For more than 10 years, major research efforts went into the search for radiation treatment conditions or additional treatments (before or after irradiation) that could modify the random mutation induction into something more specific, more directed, more economically useful (Nilan et al., 1965). Water, oxygen and time were the main factors discovered to be of influence, but their deliberate control only brought about quantitative differences, which could also be obtained from different doses, and did not really lead to any useful methodological improvement (IAEA, 1961, 1965). Later on, developing countries began to play an increasing role in mutation breeding work particularly in Asia. New varieties of rice soon appeared on the market which derived some valuable characteristics from mutation induction (IAEA, 1971; Sigurbjörnsson and Micke, 1974; Wang, 1986). In the beginning, mutation breeding was based primarily upon X-rays but now mainly gamma rays and to a smaller extent fast or thermal neutrons also started to be used.

In 1969, the joint FAO/IAEA Division started to organize course for plant breeders on the induction and use of mutation, and in the same year published the first edition of the Manual and Mutation Breeding. It may, therefore, be justified to consider 1969 as the year that marked the establishment of mutation breeding as a practical tool available to plant breeder in their endeavours to develop more productive cultivars with better resistance to stresses, pathogens and pests, and with improved quality characteristics for plant products used as food, feed or industrial raw material.
2.1. Mutation spectrum

Known mutant collections, contain only selected mutants, mostly of easily recognizable type and, therefore, not fully representative of the potential spectrum of induced mutations. A specific advantage of mutation induction, however, is the possibility of obtaining unselected genetic variation, whereas all other available germplasm has already passed screens of selection by nature or man. The question whether induced mutations duplicate the genetics variation produced by nature (Allard, 1960; Herskowitz, 1962) is rather theoretical since both natural or man-made germplasm do not represent all the possible spontaneous mutations or recombinants. When new breeding objective come up-and this will be more often in the future-it will be a matter of lucky chance if the desired variant exists among stocks in germplasm collections or inhabitats of high diversity. Spontaneous mutation rates, on the other hand, will not give much new variation to breeders. There is sufficient evidence that induced mutations fit Vavilov’s law of homologous genetic variation (Scholz, and Lehmann, 1958; Enken, 1967). It has logically been concluded that limitations of mutation breeding are not in mutagenesis as such but rather in identification and selection of desired variants (Gregory, 1956; IAEA, 1984a).

2.2. Achievements

An early record of an induced valuable mutant has been shown by Ramiah and Rao (1953). They reported about 36 X-ray induced mutations affecting different characters in rice. Of these one mutant proved useful from the economic
point of view. It had a slight shorter stature with a large number of tillers than the original parent material and proved valuable in that it performed well in rich soil where problem of lodging was serious. Looking at the progress of mutation breeding, it seems that as far as cereals are concerned major emphasis has been on obtaining mutant for improved disease resistance and improved grain quality (protein), but main results were in improving lodging resistance (short or /and stiffculm) (IAEA, 1984c; Maluszynski et al., 1986) and altering crop duration i.e. photoperiod sensitivity (Awan et al., 1982; Gottschalk and Wolff, 1983; Donini et al., 1984; Konza, 1984). Results in terms of improved grain protein were not discouraging, but remained below the rather exaggerated expectations (Micke, 1983; IAEA, 1984b; Muller, 1984; Awan and Cheema, 1988). This on one hand, is certainly due to the low heritability of quantitative endosperms characters and, the inefficient selection. With regard to disease resistance, applied selection procedures generally have been inadequate to a large extent because objectives were poorly defined due to insufficient understanding of epidemiological principles and host /parasite interaction. Nevertheless, some results have been rather spectacular (IAEA, 1977; 1983, Konza, 1984). On the other hand, it is worth noting that more than 40 years after its discovery one has begun to understand the nature of mutations in the famous ml-o locus of barley and the reasons for the universal, non specific resistance rendered by a series of recessive alleles in that locus (Jorgensen 1975, Sokou 1982). It is also the barley powdery-mildew complex where first clear experimental proof was obtained as to the

Since most mutation breeding work was performed with annual and self-pollinating cereals, most experiences relate to them. The problems in other groups of crop plants, however, are quite different. For example, in grain legumes, where breeding advances lag far behind the cereals, we have still a relatively poor adaptation of the plant architecture to modern farming systems. The plant architecture of course, being the ultimate result of numerous physiological reactions and interactions, is therefore not likely to be inherited as simply as the culm length in cereals (Micke, 1979,1984). On the other hand, reports confirm that even with single monogenic mutation a remarkable reconstruction of plant architecture is achievable in grain legumes and in other dicotyledonous plants. E.g. in chickpea (Shaikh et al., 1980), pigeon pea and mungbean (Rao et al., 1975; Khan and Siddiqui, 1996), pea (Jaranowski and Micke, 1985), castor bean (Kulkarni 1969), cotton (Raut et al., 1971; swaminathan, 1972), linseed (George and Nayar, 1973; Nayar, 1974). Fast development of computer technology enabled FAO/IAEA to organize the data base in 1987. The information contained in this data base is based on data on mutant cultivars published in various issues of Mutation Breeding Newsletter. According to the latest information available there are 1239 accessions in the FAO/IAEA Mutant Varieties Database (Maluszynski et al., 1995). These crop
varieties were developed either directly after mutagenic treatment or through crosses involving mutant varieties or mutant lines. The cumulative number of officially released mutant cultivars indicates that more than 50 percent of these varieties were released during the period between 1980-1995. Maximum numbers of crop varieties 304 have been released in China followed by India (243), the former USSR and the Russia Federation (209), the Netherlands (176), Japan (115) and USA (93). Mutant cultivars of cereal dominate (828) followed by legumes, oil crops, and industrial crops. In cereals mutation techniques were most successfully applied for improving rice (322 mutant cultivars) and barley (240) followed by wheat, maize, durum wheat and other cereals such as oat, millet, pearl etc. Application of mutation techniques for improving a particular crop or group of crops has been the subject of review papers published by the International Atomic Energy in Mutation Breeding Reviews (Hanna, 1982; Jaranowski and Micke, 1985; Daskalov, 1986; Spiegel-Roy, 1990; Robbelen, 1990; Rutger, 1992; Micke et al., 1993, Scarascia Mugnozza et al., 1993).

2.3. Chemical mutagens

Seeds and buds may be treated either in the dormant state or in the actively metabolizing stage. In the literature several methods for treating growing plants and pollen have been described.

a) Soaking in the mutagenic solution of appropriate concentration for seeds, buds and dormant cuttings.
b) A shallow cut is made in the plant stem and the mutagens applied through a wad or wick of cotton saturated in the chemical agent. This method can be used either for intact plant (Oehlker, 1943) or the developing intact inflorescence (Bianchi et al., 1961).

c) A suitable amount of the mutagen may be injected in or near the organ to be treated.

d) Although the roots are very sensitive, the mutagens in low concentration may be applied to the growth medium and allowed to enter the plant through the roots. The simple method offers the advantages of studying.

I. Chronic mutagen exposure, and

II. Sensitivity of different stages of growth and development to chemical mutagens.

e) Pollen in monolayer may be exposed to the vapour of the mutagen in a closed humid chamber (Mabuchi and Arnason, 1969).

Although chemical mutagens have been rather disappointing as compared with ionizing radiation in asexually propagated crops, it is well to compare the advantages and disadvantages of the two methods.

a) At least in sexually propagated crops, chemical mutagenesis has yielded very high chlorophyll mutation frequencies and in most instances it was more efficient than ionizing radiation with regards to mutation quantity.

b) Chemical mutagenesis is very economical due to following reason: I) a small amount of a suitable chemical mutagens II) normal laboratory glass
ware, and III) the use of a fume hood. On the other hand, when working with ionizing radiation one must have access to an X-ray machine or a more expensive gamma-rays source and must ascertain proper dosimetry of these machines. In fact, a 100 ml bottle of EMS or NMU can go along way in a plant breeders' laboratory.

c) Since most chemicals are also carcinogenic agents, extreme caution must be exercised in their use.

2.4. Alkylating agents

2.4.1. Studies with higher plants

Alkylating agents (AA) are potent mutagens can be classified broadly into monofunctional and bi- or polyfunctional ones, depending upon the number of alkyl groups present in the compound. The first chemical tested at the Indian Agriculture Research Institute was nitrogen mustard, a bi-functional alkylating agents (Bhaduri et al., 1953). However, systematic studies on different crop plant using AAs were initiated by Swaminathan and his student in the late fifties (Swaminathan et al., 1962).

From the pioneering studies of Ehrenberg and coworkers in sweden (Ehrenberg et al., 1960 and Ehrenberg et al., 1957). It was clear that AAs are particularly suited for mutagenicity studies in plants. Thus, indepth studies employing different AAs were started to correlate various biological effects, such as killing, induction of chromosomal aberrations and mutations with their chemical reaction patterns (Rao et al., 1965; Ramanna et al., 1966 and Natarajan
et al., 1966). The reactivity of AAs towards nucleophiles can be defined in terms of reaction mechanism and the dependence of reaction rates on nucleophilic strength of receptor atoms (Swain et al., 1953; Osterman et al., 1970). An useful expression of the reactivity of AAs is Swan-Scott substrate constant s, which is the measure sensitivity of AAs to the strength of nucleophilic substitution reactions have been invoked. The reaction types are generally referred to as unimolecular (SN1) and bimolecular (SN2). (Vogel and Natarajan, 1982). The ability of various alkylalkane sulfonates (such as, methyl methanesulfonate (MMS), ethyl methanesulfonate (EMS), isopropyl methane sulphonate (IPMS) to alkylate various sites in DNA was found to vary in accordance with the expectations based on s values (Swain et al., 1953). The most common adducts in DNA alkylated in neutral solution was 7- alkylguanine (Lawley et al., 1975). However, the proportional extent of reaction at the N-7 position varied according to the s values, the high s value of the AA was correlated with high N-7 alkylation. Conversely, the alkylation of 0-6 alkyl guanine was higher for AAs with low s values. The biological effects of different AAs were found to be correlated with the s values of the AA employed. For example, in barley, AA with high value s value (MMS) was found to be more cytotoxic and less mutagenic in comparison to an AA with low s value (propyl methanesulfonate, PMS) (Osterman et al., 1970). (Roa et al., 1965; Ramanna et al., 1966; Natarajan et al., 1966). Studied extensively the frequencies of chromosomes aberrations induced by different alkyl alkanesulfonates in both
mitotic and meiotic cells of barley. They found that AAs with low s values (EMS, butyl methanesulfonate –BMS and PMS) were poor inducers of chromosomal aberrations in comparison to those with high s values (MMS and methyl ethanesulfonate (MES)).(Rao et al., 1965). Higher chromosomes breaking ability of MMS in comparison to EMS was also found in studies employing root tip cells of Vicia faba (Rao et al., 1967).

2.4.2. Modification in the effect of alkylating agents in combination with DMSO

In chemical mutagenesis, secondary steps, other than the alkylation of DNA perse, are more important in the final realization of the induced mutations. This is all the more true for higher plants where multicellular systems are treated invariably. An induce alterations at the DNA level has to pass through several cellular sieves in competitions with unaffected cells (Keils, 1965; Auerbach, 1967). The fate of alkylated DNA thus depends upon the cellular processors that follow. Mutation yield and efficiency of mutagenic treatments can be considerably enhanced by manipulating the secondary factors (Kawai, 1969; Narayan et al., 1969,). Several workers have reported that DMSO acts as an useful carrier for chemical mutagens in plant (Bhatia, 1967; Gopal, 1977; Reddy and Reddy, 1979; Singh and Raghuvanshi, 1980).

2.5. Induced mutation in Vicia faba

Seeds of field bean or faba bean (Vicia faba L.) are an important source of protein in the diet of many people in countries like China, Syria, Egypt,
Ethiopia, Sudan and Morocco. On the basis of area and production, faba bean ranks fourth among pulse crops of the world, the first three being dry pea, dry bean and chick pea (Bond, 1987). In India it is cultivated throughout northern states for the sake of its broad and succulent seed pods which are used as vegetable. Its dried seeds are also used as pulse in hilly areas of U.P., H.P. and Jammu and Kashmir. Experiments at H.A.U. Hissar have revealed that faba bean out yielded chick pea, field pea and lentil (Tomar et al., 1986). Thus, a possibility exists to popularize faba bean as a new pulse crop in our country. However, the genotypes in our country have a low yield potential.

In *Vicia faba* L. there is a lack of variability for most agronomic traits. Germplasm collections from diverse sources have been made to bring all the variations at one place. The number and size of collections have increased substantially during last 10 years. The largest collection is now held by ICARDA at Aleppo, Syria. Attempts on creating new genetic variability through mutation breeding have received only limited attention in this crop.

Mutagen sensitivity in M₁ generation was worked out on several growth and yield parameters. Germination, seedling growth, pollen fertility, time taken to maturity and survival were adversely affected by the mutagens. Plant height, branching, number of leaves, pods and seeds as well as yield/plant showed varying response to different concentrations of mutagens. However, DES at all doses and EMS only at the highest dose of 0.75% had an adverse effect on these traits. Whereas, the lower doses of EMS had either no effect or
a slight promoting effect (Vandana and Dubey, 1988). In another study, 10kr of gamma rays and 0.75% DES were applied individually or in combined application, it was found that gamma rays induced more severe effects than DES (Kumar et al., 1993).

Chromosomal aberrations formed another criterion to judge the mutagen sensitivity. Vandana and Dubey (1992), Vandana (1993), Sinha and Gandhi (1994), Vandana and Dubey (1996), Bhat et al. (2005), and Prashant and Verma (2005) reported that the main types of anomalies in root tip cells were fragmentation, clumping and stickiness of chromosomes, star metaphase, giant nuclei, saucepan arrangement of chromosomes, binucleate cells, micronuclei, bridges and laggards while meiotic abnormalities included multivalent associations such as rings or chain of bivalents, fragmentation of nucleolus, precaucious separation of bivalents at Metaphase - I, single, double and multiple bridges and unequal distribution of chromosomes at the two poles at Anaphase I/II etc. The percentage of mitotic or meiotic anomalies at various stages was directly correlated to the dose of mutagen used. DES dosage inducing a higher percentage of abnormal cells than EMS (Vandana, 1993; Vandana and Dubey, 1992 and Vandana et al., 1996; Perveen, 2006). Similar anomalies have been reported by Singh Joshi (1967) and by Sjodin (1971) who investigated nearly 200 induced translocations in the species.

The frequency and spectrum of mutations in M2 generation were partitioned into those for chlorophyll, sterile and vital categories of mutations.
Among chlorophyll mutations, xantha, viridis, viridoxantha and straita types were observed while sterile mutants were flowerless, cleistogamous, fruitless and under developed seed mutants (Vandana, 1991; Fatima, 2007). Vital mutations were classified on the basis of the characters involved into mutants for cotyledonary leaf, plant height, branching, leaf, bristle, plant surface, colour and texture, floral characters, maturity period and pod and seed characters (Vandana, 1992a,b). The frequency and spectrum of chlorophyll and leaf mutation of gamma rays, EMS and nitrous oxide (N2O) seed treatment in two varieties of faba bean were studied by Yasin (1996). The frequency of chlorina type mutations was higher than that of xantah. EMS treatment was found to be most effective than the gamma rays treatment.

Mutation frequency in terms of percentage of families segregating as well as mutants/1000 M2 plants increased with an increase in concentration of chemical mutagen. DES induced higher frequency of mutations than EMS. Frequencies of vital mutations were always higher than the chlorophyll and sterile mutations (Vandana and Dubey, 1991). In the study involving gamma rays and DES applied individually as well as in combined treatment, individual DES treatment induced highest percentage of families segregating, while combined application of gamma rays and DES induced highest percentage number of mutant/1000 M2 plants (Kumar and Dubey, 1996).

Few studies have been undertaken to compare mutagenic agents for their ability to induce genetic variability in quantitative characters (Sojodin, 1971;
Enhanced variability for polygenic traits was also induced by various mutagenic treatments which was reflected by shift in mean values and increased inter and intra family variability for these traits in M2 populations. Coefficients of interfamily variability were much higher than those for intrafamily variability indicating better scope of selection between the families than within the families (Vandana and Dubey, 1990b; Vandana, 1990). A study of root of AV-8 mutant revealed a heavier nodulation in comparison to the control (Vandana and Dubey 1993). Highest phenotypic, genotypic and environmental coefficient of variability was recorded for seed yield which was closely followed by those for number of pods. Days to flower and test weight had rather small coefficient of variability (Vandana, 1992a). High heritability values for seed yield and traits like test weight, seeds/plant, seeds/pod and pods/plant have been reported in faba bean by Bakheit and Mahday (1988) and Nanda et al., (1988). On the other hand, Bond (1987) has observed that as in most legumes, yield in faba bean has low heritability because of the major effects of the environmental factors.