

Success Story of Induced Mutagenesis for Development of New Ornamental Varieties

Subodh K. Datta

Madhyamgram Experimental Farm, Acharya J C Bose Biotechnology Innovation Center, Bose Institute, 24-Parganas (N), Kolkata 700 0129, India

Corresponding author: * subodhskdatta@rediffmail.com, subodhskdatta@yahoo.com

ABSTRACT

Induced mutagenesis is well recognized as one of the most important technology for the development of new varieties through genetic manipulations. Mutation techniques, using physical and chemical mutagens, have successfully produced quite a large number of new promising varieties in different ornamental plants. This technique has been most successful in ornamental plants due to some additional advantages. Changes in any phenotypic characteristics like colour, shape or size of flower and chlorophyll variegation in leaves can be easily detected. Heterozygous nature of many of the cultivars offers high mutation frequency. The main advantage of mutation induction in vegetative propagated crops is the ability to change one or a few characters of an otherwise outstanding cultivar without altering the remaining and often unique part of the genotype. Voluminous literature for successful application of classical induced mutagenesis have been generated on radio-sensitivity, selection of materials, methods of exposure to gamma rays, suitable dose, detection of mutations, mutation frequency and spectrum, isolation of mutants and commercial exploitation of mutants. Different treatment methodology like recurrent irradiation, combined treatment, split dose, colchicine treatment, ion beam technology, space breeding, TILLING, EMAIL, etc., have been precisely determined for successful development of new varieties. The main bottlenecks in mutation breeding of vegetatively propagated plant are formation of chimeras. Therefore, attempts were made to find out the ways to overcome this situation. Management of chimera and *in vitro* technique have opened a new way for isolating new flower colour/shape ornamental cultivars through retrieval of mutated cells. Step wise advancement/refinement of practical approaches for application of classical induced mutagenesis and recent techniques for improvement of ornamental crops have been highlighted.

Keywords: biotechnological applications, chimera, genetic variation, improvement, mutagens, ornamental plants

Abbreviations: EMAIL, endonucleolytic mutation analysis by internal labeling; LET, linear energy transfer; RBE, relative biological effectiveness; TILLING, targeting induced local lesions in genomes

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INTRODUCTION

A number of plant breeding methods like cross-breeding, induced mutagenesis and molecular breeding are available for crop improvement and more specifically to develop new varieties. Induced mutagenesis is now an established method for crop improvement. The use of induced mutations has over the past 50 years played a major role in the

development of superior crop varieties translating into a tremendous economic impact on agriculture and food production. Approximately 3000 varieties have been released worldwide that have been derived either as direct mutants or from their progenies. Officially released mutation-derived varieties include many important crops such as rice, cotton, rapeseed, sunflower, sesame, grapefruit, banana, ornamentals, etc. and they have made a major global

economic impact. Mutation-derived varieties with changed traits have resulted in a synergistic effect on increasing the yield and quality of the crop, improving agronomic inputs, crop rotation, and consumer acceptance. Mutation techniques have become a major tool for breeding ornamental plants. Detailed review on the global impact of mutation derived varieties developed and released all over the world have been published (Ahloowalia *et al.* 2004; Lagoda 2009; Kharkwal and Shu 2009; Jain 2010). The main advantage of mutation induction in vegetative propagated crop is the ability to change one or a few characters of an otherwise outstanding cultivar without altering the remaining and often unique part of the genotype (Broertjes 1968). Mutation breeding has become more successful in ornamental plants due to some additional advantages. Changes in any phenotypic characteristics like colour, shape or size of flower and chlorophyll variegation in leaves can be easily detected. Heterozygous nature of many of the cultivars occur high mutation frequency.

A large number of new promising varieties have been successfully developed in different ornamental plants by mutation techniques using both ionizing radiations and other mutagens. Attempts are made, in the present chapter, to highlight the knowledge concerning the advancement/refinement of practical approach for application of induced mutagenesis for improvement of ornamental crops. Emphasis has been given to elaborate some of the interesting findings of classical mutation breeding and step wise refinement of mutation technique for better result. Voluminous literature is available in the field of application of mutagenesis for improvement of ornamental plants. The details of prospects, utilization of induced mutations, list of released mutant varieties in different crop plants and many important aspects of mutagenesis in vegetatively propagated ornamentals have been compiled and published as review papers (Smith 1958; Stube 1959; Khoshoo 1968; Broertjes 1969; Sigurbjorsson and Micke 1969; Swaminathan 1971; Broertjes and Van Harten 1978; Gottschalk and Wolff 1983; Micke *et al.* 1987, 1990; Bhatia 1991; Micke 1991; Kawai and Amano 1991; Wang 1991; Anonymous 1994; Maluszynski *et al.* 1995; Datta 1988, 1997a, 1997b; Schum and Preil 1998; Maluszynski *et al.* 2000; Datta 2001, 2004; Ahloowalia *et al.* 2004; Datta and Teixeira da Silva 2006; Jain 2006; Datta 2009a, 2009b; Nagatomi and Degi 2009; Datta 2010; Jain 2010). Appreciable informations have been accumulated on both applied and basic aspects like radiosensitivity, selection of material, methods of exposure to gamma rays, suitable dose of gamma rays, colchicine treatment, recurrent irradiation, detection and isolation of mutants, commercial exploitation of mutant, etc. Creation of genetic variability is pre-requisite for development of new variety. Induced mutagenesis is an established method for plant improvement using physical and/or chemical mutagens. For improvement programme, one should have all up-to-date knowledge about technical details and their merits and demerits. Breeder should be aware of the potential and the limitations of various approaches and should deliberately choose the strategy which is most appropriate as well as economic for reaching the aims under prevailing circumstances of variety improvement.

CLASSICAL INDUCED MUTATION

The concept of using induced mutagenesis for crop improvement using different physical and chemical mutagens developed dates back to the beginning of the 20th century. First observation on effects of radium rays on higher plant was reported by Gager (1908). Study of induced artificial genetic changes commenced in 1927 by Muller who discovered the mutagenic property of X-ray in *Drosophila* and similar mutational effects was demonstrated by Stadlar (1928a, 1928b) in plants (*Zea mays* L. and *Hordeum vulgare* L.) by X-ray and radium. In the same year mutation has also been induced in *Nicotiana* by Goodspeede and Olsom (1928). They assumed that induced genetic changes

(induced mutation) could play an important role in future genetic improvement of plants.

Induced mutagenesis work was concentrated at early stage mainly on fundamental research i.e. detection of new mutagens and their effects on living cells. Slowly more emphasis was given on experimental mutagenesis for better understanding the mechanisms of mutagenesis (Grabowska and Mynett 1970; Gottschalk and Wolf 1983; Veleminsky and Gichner 1987). Practical mutation breeding procedure by use of mutagenesis strictly for plant breeding developed after publications of Freisleben and Lein (1943a, 1943b) in Halle (Germany). They successfully induced mildew resistance in barley and developed a practical mutation breeding procedure. Information's accumulated on optimal treatment doses, treatment condition, mutation frequency and mutation spectra after systematic studies in Sweden using X-ray by Hoffman (1959). High potential for bringing genetic improvement by induced mutation was realized. From 1960 and onwards developing countries began to play an increasing role in mutation breeding work.

Some other types of ionizing radiations, e.g., gamma rays, neutron, radioisotopes emitting beta and alpha rays were also included for radiobiological studies. After these discoveries, the effect of radiations on chromosome and genes was the main aspect of study. In course of time, the subject 'radio sensitivity' has been developed. It deals with the sensitivity of plant or animal tissues to radiations. Radio sensitivity studies may lead to an understanding of the mechanism of action of ionizing radiations. Such studies also provide valuable information on radiation protection and radiation therapy. Determination of radio sensitivity of any crop is pre-requisite for large scale irradiation of materials for induction of mutation. A number of workers have studied the cyto-morphological and physiological effects induced by ionizing radiations in higher plants. Studies indicated that various biological, physical and chemical factors (genotype, stage of cellular development, chromosome number, age of the tissue, storage after irradiation, oxygen, chemicals, water, temperature, ionization density, combined treatment, etc.) can modify the effects of radiation in plants (Sagawa 1957; Sparrow *et al.* 1968; Datta 1984, 1992a, 2006a). According to available literature the radio sensitivity is known to depend on both nuclear factors (interphase chromosome volume, interphase nuclear volume, chromosome number, chromosome size, nucleolus and heterochromatin, centromere number and position, degree of polyploidy and nuclear DNA content) and extra nuclear factors (cytoplasmic factors, moisture content of seed coat and seed weight). Induced chromosomal aberrations as the end point of radio sensitivity was studied in root meristems and found that different ornamental cultivars were differently sensitive irrespective of flower colour, size and shape and it has been clearly mentioned that radio sensitivity in the garden chrysanthemum is a genotype dependent mechanism (Datta 1992b; Banerji and Datta 1993).

Determination of suitable dose of gamma radiations for the induction of somatic mutation is most essential. The working dose of some common ornamentals has been precisely determined (Datta 2009a). The frequency of mutation varies with the cultivar and dose of gamma rays. Some cultivars are moderately sensitive, some are more sensitive and some are resistant to mutagens. Like mutation frequency, spectrum of mutation also varies with the cultivar and dose of gamma rays.

Since the 1930s mutation induction has been applied to ornamental plants. De Mol released the first officially registered commercial mutant cultivar 'Faraday' using X-ray induced mutation in *Tulipa* sp. cv. 'Fantasy' in 1936 (reported in 1945). The most successful story of mutation breeding is "Horim" group of chrysanthemum mutants in the Netherlands, which in 1979 took as much as 35% of the total Dutch market of 500 million plants (Broertjes *et al.* 1980).

Mutation breeding, in the beginning, was based primarily upon X-rays, but now mainly gamma rays (gamma rays have induced about 70% of the world's mutant vari-

eties) and to a smaller extent fast or thermal neutrons also started to be used. Detrimental effects of ionizing radiations motivated a number of researchers to use chemical mutagens and more and more chemicals were identified as possessing mutagenic properties. Several chemicals were found to be more powerful in terms of high mutation rates than ionizing radiation (Roebelen 1959; Ehrenberg *et al.* 1961; Auerbach 1961; Konzak *et al.* 1965; Rapoport *et al.* 1966). Various chemical mutagens give higher mutation rates; some produce a higher ratio of gene mutations vs. deletions or other chromosome mutations. However, there are several practical problems with chemical mutagens (soaking seeds, penetration to the relevant target cells, safety of handling and disposal, poor reproducibility, persistence of the mutagen or its metabolites), which may compensate the advantages (FAO/IAEA 1977). But the optimistic expectations soon faded away and chemical mutagens were considered to be one of several means for inducing genetic variation.

Ornamental plants comprise diverse groups including flowering and foliage pot plants, cut flower crops, tuber and bulb crops, annual and perennial garden plants as well as trees and shrubs. Mutation induction techniques have been applied to all these groups. The application of mutation induction techniques has been limited in seed propagated ornamentals as compared to vegetatively propagated species. Induced mutations in ornamentals comprise traits, such as altered flower characters (colour, size, morphology, fragrance); leaf characters (form, size, pigmentation); growth habit (compact, climbing, branching) and physiological traits such as changes in photoperiodic response, early flowering, free-flowering, flower keeping quality, and tolerance to biotic and abiotic stresses.

The use of induced mutations has over the past 50 years played a major role in the development of superior crop varieties resulted in the official release of over 3000 new crop varieties in some 170 species including both seed and vegetatively propagated crops. Classical induced mutagenesis has successfully produced quite a large number of new varieties in different ornamental plants. Total number of new mutant ornamental varieties developed throughout the world is available in IAEA, Vienna Mutant Database. However, few important references are mentioned on some important ornamentals as a ready reference. Attempts have been made to highlight different important basic aspects which may be helpful as guideline for large scale mutagenesis work on ornamental crops. Broertjes (1976) studied effects of mutagenesis on auto-tetraploid *Achimenes*. Broertjes (1971) also studied effects of fractionated dose and different basic aspects of radiation-induced changes in African violet. Broertjes and Verboom (1974) treated young rhizomes of diploid and triploid cultivars of *Alstroemeria* and detected solid mutants. A number of mutants with changed flower colour were detected after irradiation in *Amaryllis* (*Hippeastrum*) (Kaicker and Singh 1979). Higher mutation frequency and spectrum and solid mutants could be induced after irradiating leaves of triploid hybrids of *Begonia* (Doorenbos and Karper 1975; Doorenbos 1973). Several mutants have also been developed after irradiation and commercialized in *Begonia* (Matsubara *et al.* 1975; Mikkelsen *et al.* 1975; Harney 1976; Molnar 1976; Anonymous 1977; Lin and Molnar 1983). The suitable dose of gamma rays for irradiation of stem cuttings of *Bougainvillea* has been standardized from 250 to 1250 rads (Datta 1992c). Radiosensitivity of large number of *Bougainvillea* cultivars to gamma rays has been determined for large scale induced mutagenesis experiments (Jayanthi *et al.* 1999; Srivastava *et al.* 2002). Four gamma ray induced mutants have been released by Abraham and Desai (1977). Most promising and beautiful chlorophyll variegated mutants 'Arjuna', 'Pallavi', 'Mahara Variegata', and 'Los Banos Variegata' induced after gamma irradiation have been commercialized (Gupta and Nath 1977; Datta and Banerjee 1990, 1994). For induction of mutation X- or gamma-rays (5-30 Gy) has been used and their effects on rhizomes of *Canna* cultivars with different

ploidy level have been reported (Nakornthap 1965; Gupta 1966; Mukherjee and Khoshoo 1970; Desai and Abraham 1974). Induction of somatic flower colour mutation in carnation was first reported by Richter and Singleton (1995). Induced mutation frequency and other morphological alterations in carnation using medium to a high radiation dose created interest on mutagenesis work in ornamentals (Sagawa and Mehlquist 1956, 1957, 1959; Sagawa 1957; Mehlquist and Sagawa 1962; Searnaaij and Demmink 1970, 1971; Searnaaij 1974; Sparnaaj *et al.* 1974; Richter and Singleton 1995). Voluminous work has been done on *Chrysanthemum morifolium* Ramat. for its improvement through induced mutagenesis using a wide range of physical and chemical mutagens (Datta and Gupta 1980, 1983; Broertjes and Van Harten 1988; Datta 1988; Shukla and Datta 1993; Datta 2001; Teixeira da Silva 2003; Datta and Teixeira da Silva 2006). Early reports indicated that some of the cultivars withstood 3000R x-rays and the optimum dose lay between 2000-4000 rad (Jank 1957a, 1957b; Sheehan and Sagawa 1959; Fuji and Mabuchi 1961; Bowen *et al.* 1962; Dowrick and El-Bayomi 1966a, 1966b). Some authors, however, used higher doses like 8-25 Krad gamma rays (Cawse 1966; Yamakawa and Sekiguchi 1968; Broertjes 1966). The optimum dose of gamma rays for inducing mutations has been reported to be 1.5 to 2.5 Krad for small flowered chrysanthemum (Datta 1990a, 1992a, 1992b). Radiosensitivity of chrysanthemum has been very critically determined on the basis of chromosomal aberrations as the end point, flower colour, size and shape, chromosome number, Interphase Nuclear Volume (INV), Interphase Chromosome Volume (ICV) and 2c DNA content (Datta and Banerji 1991; Datta 1992a; 2001; Banerji and Datta 1993; Yamaguchi *et al.* 2008). It has been very clearly determined that radiosensitivity in the garden chrysanthemum is a genotype dependent mechanism. Experimental results have proved that all colours of chrysanthemum including white and yellow are mutable (Jank 1957; Bowen 1965; Datta 1985). Several mutations were detected after irradiation of young tubers of cyclamen (Breider 1959). Variegated and dwarf-type mutants have been reported in *Coleus* after treating cuttings with gamma rays and fast neutron (Love and Mullenax 1964; Love and Constantin 1966; Love and Malone 1967). A number of flower colour mutants have been developed and commercialized in *Dahlia variabilis* using radiation (Grabowska and Mynett 1964; Broertjes and Ballego 1967, 1968; Lantin and Decourtye 1970; Das *et al.* 1974, 1975, 1977, 1978; Dube *et al.* 1980). Rooted cuttings of *Euphorbia splendens* were treated with gamma rays and mutants with changed leaf form and colour were detected (Koo and Cuevas-Ruiz 1974). Both physical and chemical mutagens have been successfully used for the induction of mutation in *Gladiolus*. Physical mutagens like gamma rays, X-rays, fast neutron, thermal neutrons and electric shock and chemical mutagens like aluminium chloride, colchicine, diethyl sulfate, formaline, glycol, methyl methanesulfonate (MMS), dimethyl sulfate, ethyl methanesulfonate (EMS), nitroso dimethyl urea (NDMU), *N*-nitroso-*N*-ethylurethan (NEU) and *N*-nitroso-*N*-methylurethan (NMU) were used for mutation studies which have already been reviewed (Buiatti and Tesi 1968; Banerji *et al.* 1981; Banerjee and Datta 1987, 2001). Mutants with changed flower colour and form were induced in 'Alipur Beauty' and 'Cruentus' cultivars of hibiscus (*Hibiscus rosa-sinensis*) using semi-acute gamma rays (Das *et al.* 1974, 1977). Five petalled single flower type mutant has been induced in double flower type cv. 'Alipur Beauty' treating stem cuttings with 4 krad gamma rays (Banerjee and Datta 1986). EMS induced dwarf mutant has been induced in *Impatiens platypetala* after treating seeds with EMS (Weigle and Butler 1983). X-ray was successfully used for inducing mutation in *Kalanchoe* (Johnson 1948; Broertjes and Leffring 1972; Nakornthap 1973, 1974). Izuka and Ireda (1963, 1968) studied effects of X-ray on different characters of *Lilium formosanum*. Mutants with changed flower colour and low light tolerant have been induced by treating bulb scale of different *Lilium* hybrids

(Broertjes and Alkema 1970). Stem cuttings of *Perennial portulaca* were treated with gamma rays for induction of mutation (Banerjee 1967; Cotter 1963; Gupta 1966, 1970; Lata and Gupta 1971; Desai 1974; Abraham and Desai 1978; Raghuvanshi and Singh 1979; Tangsombatvichit *et al.* 2008). Seven mutant varieties have been released (Gupta 1966; Desai 1973, 1974). Mishra and Raghuvanshi (1986) used gamma rays, EMS and combined treatment and detected different variants affecting different floral characters and isolated stable mutants with changed flower colour in *Portulaca gradiflora*. Younis and Borham (1975) used 500 to 3000 rad of gamma rays and induced genetical and morphological variability in *Polianthes tuberosa* L. Abraham and Desai (1976) used X-rays, neutron and gamma rays separately and their combinations and studied their effects on vegetative characters and flower of single and double type tuberose. Patil *et al.* (1975) induced one large flower mutant using 0.5 Krad gamma rays. Two chlorophyll variegated mutants using 2.5 krad gamma rays have been developed and released (Gupta *et al.* 1974). Extensive studies have been carried out on *Tulipa* mainly towards bulb size, ploidy level and optimum working dose for inducing mutations (Matsuda 1942; Myodo 1942; De Mol 1949; Nybom 1961; Matsubara *et al.* 1965; Nezu 1965; Grabowska and Mynett 1970; Custers *et al.* 1977; Matsubara 1982; Broertjes and Van Harten 1988). Induced mutagenesis work has been very successful in rose and quite a large number of new varieties with changed flower colour and shape and growth habit of plant have been developed using both physical (x-ray, gamma ray) and chemical (EMS, NMU, EI) mutagens (Nakajima 1965, 1970, 1973; Chan 1966; Domergues *et al.* 1967; Streitberg 1967; Kaicker and Swarup 1972; Usenbaev and Imankulova 1974; Gupta and Datta 1982; Gupta *et al.* 1982; Kaicker 1982, 1986; Datta and Gupta 1984a, 1985a Kaicker and Dhyani 1985; Huang and Chen 1986; Datta 1986a, 1986b, 1987b, 1989, 1997b). Datta (1986b) used recurrent gamma irradiation for induction of mutation in rose. He found cumulative effects on sprouting, survival and plant height. Percentage of somatic mutations and spectrum of mutations were higher after recurrent irradiation in comparison to single irradiation (Datta 1994; Datta and Gupta 1985a). Colchicine for the first time has been used to induce flower colour mutations in rose (Gupta and Datta 1983; Datta and Gupta 1985b). Year-round flowering mutant has been reported in *Streptocarpus* (Brown 1974; Davis and Hedley 1975; Van Raalte and Van Raalte-Wichers 1974).

Recurrent irradiation

Recurrent irradiation means irradiating plant materials that had already been irradiated in one or more subsequent generations for expanding more genetic variability which otherwise is not possible through single irradiation. For accumulating and expanding genetic variability, use of recurrent irradiation in breeding programme has been proposed long back (Freisleben and Lein 1943a, 1943b; Khadr and Frey 1965; Brock and Shaw 1969; Micke 1969; Walther 1974). Recurrent irradiation experiment with chrysanthemum and rose result more genetic variability and increased percentage of mutations and spectrum of mutations. It is advised that recurrent mutagen treatment may provide an even greater range of genetic variability than would a single mutagen treatment in vegetatively propagated ornamentals. This method can be successfully used in routine mutagenesis programme for inducing novelties in flower colour/shape (Datta 1986a, 1986b, 1991).

Colchicine as a mutagen

Colchicine has been used for a long time as a polyploidizing agent. Normally in colchiploidy breeding attention is paid to chromosome duplication and its effects on phenotype. Much attention has not been paid to mutations through colchicine treatment, which has already been

possible in several crop plants (Datta 1976, 1990b). Colchicine has been successfully used for inducing somatic flower colour mutations in chrysanthemum (Datta and Gupta 1984b, 1987) and rose (Gupta and Datta 1983; Datta and Gupta 1985b). A colchicines-induced mutant of chrysanthemum has been released in the name of Colchi Bahar (Datta 1987b). It may be pointed out that normally after colchicine treatment attention is paid to chromosome duplications and its effect on phenotype. When there is no polyploid formation and when there is no gigantism in desired characters in induced polyploid in particular taxa, colchicine breeding is through to be unsuccessful. But careful observations have led to the understanding that although colchicine is known more familiarly as a polyploidizing agent, it may also be used as a very good mutagen (Datta 1990b).

Combined treatment

Greater stress is now given to the use of combined treatment of physical and chemical mutagens. It has been shown that radiation-induced genetic damage and mutation frequencies can be modified and influenced by treatment of seeds with chemicals before or after irradiation (D'Amato and Gustafsson 1948; Gustafsson and Nybom 1949; Ehrenberg *et al.* 1952; Gaul 1958; Bose and Banerjee 1968; Sree Ramulu 1971; Bose and Maiti 1972; Jana *et al.* 1974; Killion and Constantin 1974). Such modifications of mutation process may open the way for directing and controlling the production of desirable mutants in ornamentals (Misra and Raghuvanshi 1986; Datta 1997a).

INDUCED MUTAGENESIS IN CROSS BREEDING

It has been realized that use of induced mutagenesis in various cross-breeding programme is more important than the direct use of mutants and the number of mutant cultivars steadily increased from cross-breeding combinations involving induced mutants. Various possible cross-combinations like 'crossing the mutants with the original parents variety or line', 'crossing different mutants from the same parent line', 'crossing different mutants from different parent lines', 'crossing the mutants with a different variety or line', 'crossing two varieties apparently carrying the same mutant' etc. have been recommended (Micke 1968, 1969; Romer and Micke 1974; Micke *et al.* 1985; Datta 2005).

ACUTE AND CHRONIC IRRADIATIONS

Experimental results indicate that plants are differentially sensitive to acute (gamma chamber/room) and chronic (gamma field) radiation separately and in combination of both methods. Chronic irradiation has resulted in maximum mutation frequency and spectrum in chrysanthemum over acute irradiation (Nagatomi and Degi 2009). This technique has been successfully applied and developed a number of useful mutant varieties in ornamentals and other crops (Broertjes 1971; Nagatomi 1991; 1992; 2002; Nagatomi *et al.* 1993; Richter and Singleton 1995; Nagatomi *et al.* 1996).

IN VITRO ADVENTITIOUS BUD TECHNIQUE

Many plants can be propagated by different types of adventitious plantlets. Adventitious buds can be stimulated either on roots (*Phlox*), bulbs (*Hyacinthus*) or leaves (*Begonia*, *Saintpaulia*, *Streptocarpus*) or bulb scales (*Lilium*). The most important but disadvantageous result is that the majority of the adventitious shoots proved to be of a chimeral nature and obviously develop from more than one cell. Technique has been standardized for development of *in vitro* adventitious bud using different types of explants. This technique was found to produce solid mutants in different vegetatively propagated ornamentals like *Kohleria* (Geier 1988), *Achimenes* (Broertjes 1973), *Begonia* (Brown and Harney 1974; Mikkelsen and Sink 1978; Roest *et al.* 1981; Broertjes 1982a), *Chrysanthemum morifolium* (Broertjes

and Roest 1976; Broertjes *et al.* 1976), *Gerbera jamesonii* (Jerzy and Lubomski 1991), *Kalanchoe* (Broertjes and Leffring 1972; Nakornthap 1974; Shama Rao and Singh 1976; Shama Rao 1977; Karper and Pierik 1981; Van Dordrecht 1984), *Pelargonium* (Grunewaldt 1983), *Saintpaulia* (Broertjes 1972), *Streptocarpus* (Broertjes 1969, 1982b), etc. About 350 plant species belonging to various plant families were detected to develop adventitious plantlets (Broertjes *et al.* 1968). Adventitious bud technique in which the buds develop directly from one or a restricted number of epidermal cells was found to be very useful in producing mutants which are either solid or have large mutated sectors. But the application of this technique is limited.

DETECTION OF MUTATIONS

Somatic mutations in vegetatively propagated plants are mostly detected in first vegetative generation M_1V_1 . Reports are also available that mutations have been detected in M_1V_2 , M_1V_3 and later vegetative generations from normal looking irradiated plants in M_1V_1 (Buiatti and Tesi 1968; Das *et al.* 1974; Usenbaev and Imankulova 1974; Gupta and Jugran 1978; Datta 1992a). It has been observed that chances of getting solid mutants are more in M_1V_2 and later generations. Screening for mutations should not be confined to M_1V_1 only, but it should be continued in M_1V_2 and subsequent vegetative generations. The mutated cell expresses its mutant character if it gets chance to express in M_1V_1 . The mutated cells of the lower auxiliary buds remain in the dormant stage and express its mutant character when included during vegetative propagation in M_1V_2 (Datta 2001).

CHLOROPHYLL VARIATION

Chlorophyll variegated leaves provide additional beauty to the plants at the time of blooming and even when there is no flower. Plants with variegated leaves have considerable economic importance in floriculture trade due to their decorative foliage (Datta 1998, 2006b, 2009c). A number of promising chlorophyll variegated mutants have been developed through gamma irradiation and commercialized in bougainvillea (Abraham and Desai 1977; Banerji and Datta 1987; Datta and Banerji 1990, 1994; Datta 1992c) and *Lantana depressa* (Datta 1995).

MUTATION IN FLOWER MORPHOLOGY

Radiation induced phenotypic variation including several interesting changes in flower form for novelties have been reported. A number of interesting morphological changes in flower forms have been reported after treatment of seeds of annual chrysanthemum with x-ray doses (Jain *et al.* 1961; Rana 1964, 1965). Commercial varieties with interesting changed flower morphology have been released in *Portulaca* (Gupta 1979), *Begonia*, *Chrysanthemum*, *Petunia* and *Hyacinthus* (Broertjes 1966; Broertjes and van Harten 1988) and carnation (Simard *et al.* 1992; Cassels and Walsh 1993). A single flower form mutant has been developed after treatment with gamma rays in double flower type *Hibiscus* cv. 'Alipur Beauty' (Banerji and Datta 1988). Series of mutants with changed flower type have been developed in chrysanthemum after gamma irradiation (Datta and Gupta 1984c; Datta *et al.* 1985; Datta 1990c; Banerji and Datta 1992; Datta and Banerji 1993; Banerji and Datta, 2002, 2003). A mutant with altered plant morphology was detected in *Torenia fournieri* (Sawangmee *et al.* 2011).

MUTANT OF A MUTANT

Mutant genotypes can be further improved through mutation and new mutant characters can be developed. Geier (1988) treated mutant Kohleri with N-nitrosomethylurea (NMU) and induced a compact type mutant. Broertjes *et al.* (1980) were successful to develop hundreds of mutants by successive use of radiation induced mutants of chrysanthe-

mum cv. 'Horim'. Datta (1985, 1996) used mutant genotypes (gamma ray induced mutants) of chrysanthemum and developed new flower colour mutants. Datta and Shukla (1996) treated bulbs of two gamma ray induced chlorophyll variegated mutants ('Rajat Rekha' and 'Svarna Rekha') of *Polianthes tuberosa* L. with 500 and 1000 rads of gamma rays and successfully induced new pattern of chlorophyll variegated mutants.

IN VITRO MUTAGENESIS AND MANAGEMENT OF CHIMERAS

The main bottlenecks in mutation breeding of vegetatively propagated plants i.e. treatment (physical and/or chemical mutagens) of bulbs, tubers, rhizomes, cuttings/suckers, other plant parts or whole plants all having buds with multicellular apices composed of a number of fairly autonomous cell layers, automatically leads to the formation of chimeras. In multicellular organisms, after irradiation of a multicellular apex, such mutated cell is exposed to the so called diplontic selection i.e. the competition between the mutated cell and the surrounding non-mutated ones. The mutated cell develops a group of cells and finally a cell layer. The final result of a diplontic selection is a low number of mutated plants and a restricted mutation spectrum (Gaul 1961). A large number of chimeric new flower colour/shape mutants are lost every year from mutagenesis experiments. Therefore, concept of *in vitro* mutagenesis developed which has opened new possibilities for inducing increased number of mutants and solid mutants. The main advantage of this technique is to overcome chimera formation. *In vitro* mutagenesis experiments can be conducted with large population, within limited space and any time of the year. The chance of getting solid mutant is more in *in vitro* mutagenesis. Protocol has already been standardized for *in vitro* regeneration of chrysanthemum (Ben Jacob and Langhans 1972; Earle and Langhans 1974; Lu *et al.* 1990; Malaure *et al.* 1991a, 1991b; Nagatomi *et al.* 1993). The main advantage of this method is that it helps to avoid chimera formation in the M_1V_1 (Maliga 1984; Ahloowalia 1995; Maluszynski *et al.* 1995). Efficient technique has been standardized by the author and his team for direct shoot regeneration from individual floret of chrysanthemum and a number of new flower colour/shape mutations have been isolated through management of induced and spontaneous mutant chimeric tissues (Chakrabarty *et al.* 1999, 2000; Mandal *et al.* 2000a, 2000b; Datta *et al.* 2001). A series of papers have been published on *in vitro* mutagenesis experiments on *Saintpaulia* and *Pelargonium* (Skirvin and Janick 1976; Grunewaldt 1983, 1988), carnation (Johnson 1980; Simard *et al.* 1992; Cassels and Walsh 1993), chrysanthemum (Jung-Heiliger and Horn 1980; Preil *et al.* 1983; Huitema *et al.* 1986; Dalsou and Short 1987; Huitema *et al.* 1989; Jerzy 1990; Jerzy and Zalewska 1996; Schum and Preil 1998; Mishra *et al.* 2003; Datta and Mandal 2005), *Eustoma grandiflorum* (Nagatomi *et al.* 1996), *Gerbera* and *Rosa* spp. (Walther and Sauer 1986a, 1986b; Laneri *et al.* 1990; Jerzy and Lubomski 1991; Jerzy and Zalewska 1992).

ION BEAM TECHNOLOGY

Ion beam technology was found to show high relative biological effectiveness (RBE) compared to low linear energy transfer (LET) radiation such as gamma rays, X-rays and electrons (Blakely 1992) and basic research on plant mutation by ion beams began in 1991 (Goodhead 1995; Nakai 1995; Feng *et al.* 2006, 2009). Ion beam, as a new mutation technique, has been widely used in mutation breeding, and great achievements have been made in agriculture (Watanabe 2001; Okamura 2006; Tanaka 2009; Tanaka *et al.* 2010). Effects of ion beams have been investigated on several plants including few ornamentals like chrysanthemum (Nagatomi *et al.* 1996; Ueno *et al.* 2002, 2004; Ikegami *et al.* 2005; Matsumura *et al.* 2010), carnation (Okamura *et al.* 2003), petunia (Okamura *et al.* 2006), rose (Yamaguchi *et*

al. 2003), *Torenia* (Miyazaki *et al.* 2006; Sasaki *et al.* 2008), *Lotus* (Oka-Kira *et al.* 2005) and *Cyclamen* (Sugiyama *et al.* 2008), etc. Ion beam technology has been further modified as ion beam implantation into organisms. Ion implantation could become a new mutation technique. The interactions between the implanted ions and complicated organisms have been studied intensively (Yang *et al.* 2007). They have proposed that a combination of energy absorption, mass deposition, and charge transfer of energetic ions in the seeds resulted in the biological effects. It has been reported that targeted ion implantation of shoot apical meristems of *Arabidopsis* embryos induces long-distance systemic effects on root apical meristems (Feng *et al.* 2009). The implantation of ions with different mass could lead to biological effects at different levels. The energies of the ion-induced fragment ions and emitted electrons are particularly important in the mechanism of long-distance damage in ion implantation of an organism. If the energy of emitted fragments is sufficiently high, these fragments will induce further damage to surrounding molecules. Since the biological organisms are not good electrical conductors, the accumulated surface charge is not immediately released. The long term accumulation of surface charge not only affects the electrical characteristics of biological organisms, but also may lead to changing an electrostatic field across the cellular membrane, influencing various biochemical processes (Feng *et al.* 2009). It has been reported that targeted ion implantation of the shoot apical meristem of *Arabidopsis* embryos induced damage to the root apical meristem indicating long distance systemic effects in intact organisms. Similar to other radiation, there are many coexisting factors, including energy, mass and charge of the implanted ions, having an effect on biological effects, making it difficult to distinguish between direct and indirect effects of these factors (Schlatholter *et al.* 2005; Feng *et al.* 2006; Huang *et al.* 1996; Yang *et al.* 2007; Feng *et al.* 2009).

SPACE BREEDING

Space breeding has been set up to enhance genetic diversity to breed new crop varieties (Halstead and Dutcher 1987; Dutcher *et al.* 1994; Liu and Zheng 1997; Mei *et al.* 1998; Liu *et al.* 2000, 2004, 2007). Strong cosmic radiation, microgravity, weak geomagnetic fields, and a hyper-clean super-vacuum are the main characteristics of the aerospace environment. Experimental results showed that space conditions are mutagenic. It affects on seed germination and plant growth and induces cytological aberrations. The frequency of morphological and cytological aberrations was higher when seeds were kept for longer time in space (Halstead and Dutcher 1987; Gu and Shen 1989; Mei *et al.* 1998). Combined effects of both cosmic radiation and microgravity are the main causes of the genetic changes. Strong vibration and blast force associated with spacecraft launch and landing also play as casual agents contributing to the increased frequency of chromosomal aberrations during space flight (Halstead and Dutcher 1987; Liu and Zheng 1997; Gu and Shen 1989). China has conducted space-induced mutagenesis over 2,000 accessions of plant seeds belonging to 133 species using recoverable satellites, Shenzhou spacecrafts and high-altitude balloons (Liu *et al.* 2007) and approved, approximately, 66 new varieties developed by the space-breeding programme (Qiu *et al.* 1998; Shi *et al.* 2000; Liu *et al.* 2005). Space-induced mutation can be a novel and effective way to enhance genetic diversity from which to breed new crop varieties. Because of the need for major investment and technological support, the chance of space flight experimentation is very limited. Therefore, sustainable progress and future perspective of space-induced mutagenesis for crop improvement depends upon international cooperation (Liu *et al.* 2009).

TARGETING INDUCED LOCAL LESIONS IN GENOMES (TILLING)

Targeting Induced Local Lesions in Genomes (TILLING) is one of the high-throughput, non-transgenic reverse-genetic approaches technique combining chemical mutagenesis with a sensitive DNA screening-technique that enables the recovery of individuals carrying allelic variants at candidate genes (McCallum *et al.* 2000a, 2000b; Ostergaard and Yanofsky 2004; Slade and Knauf 2005; Slade *et al.* 2005; Alonso and Ecker 2006; Till *et al.* 2009; Wang *et al.* 2009). TILLING combines traditional mutagenesis followed by high-throughput mutation discovery which can improve the efficiency of using induced mutations to develop crops with improved traits (McCallum *et al.* 2000a; Colbert *et al.* 2001). It is an efficient early-screening tool for specific point mutations in genes of interest from a small population and enables geneticists to analyze gene function and associate genotype with phenotype. It is useful in scanning gamma-irradiated mutant populations (Sato *et al.* 2006). The technique is yet to be applied to ornamental crops.

ENDONUCLEOLYTIC MUTATION ANALYSIS BY INTERNAL LABELLING (EMAIL)

For detecting the rare mutations in specific genes in pooled samples, an improved technique 'Endonucleolytic Mutation Analysis by Internal Labelling' (EMAIL) has been developed using capillary electrophoresis which offers an increased degree of sensitivity. This technique is highly improved over TILLING approach and offers the plant breeder a new tool for efficient screening of induced point mutation at an early stage for variants in genes of specific interest before taking plants to field trial (Cordeiro *et al.* 2006; Cross *et al.* 2008; Lee *et al.* 2009). This new technique offers an increased degree of sensitivity in detection and provides information to assist the molecular characterization of mutations in specific genes of interest. The technique is yet to be applied to ornamental crops (Lee *et al.* 2009).

CONCLUSION

From the present review it is very clear that majority of commercial varieties of ornamental species have been developed through conventional breeding, induced mutation and selection. Induced mutagenesis at its present status appears to be well standardized, efficient and cost effective. Although mutation breeding is a random (chance) process, reports are available for directive mutation (Datta 1990a, 1990d, 2001, 2005). At this stage it is possible to increase the rate of mutant development by combining the classical mutation breeding and advanced technique. This concept has been clearly proved by the management of chimera through direct regeneration from petals of chrysanthemum (Chakrabarty *et al.* 1999, 2000; Mandal *et al.* 2000a). Classical mutagenesis combined with management of chimera and *in vitro* mutagenesis are now well standardized and most promising techniques for development of new and novel varieties (Datta and Chakrabarty 2005).

ACKNOWLEDGEMENTS

Thanks are due to The Director, National Botanical Research Institute, Lucknow for providing facilities for doing experimental research on induced mutagenesis. The author thankfully acknowledges the Council of Scientific and Industrial Research, New Delhi for providing an Emeritus Scientist Fellowship. Thanks are due to the Director, Bose Institute, Kolkata for providing present working facilities. The author thanks Dr. Jaime A. Teixeira da Silva for making significant improvements to language, content and style.

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