Success Story of Induced Mutagenesis for Development of New Ornamental Varieties

Subodh K. Datta

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 has 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

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-derivated varieties include many important crops such as rice, cotton, rapeseed, sunflower, sesame, grapefruit, banana, ornamentals, etc. and they have made a major global

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

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

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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 (Ahlouwalia et al. 2004; Lagoda 2009; Khosho 2009; Jain 2009; Datta 2009a, 2009b; Datta et al. 2010). Induced 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 color, shape or size of flower or 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; Khosho 1968; Broertjes 1969; Sigurbjörsson 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; Ahluwalia et al. 2004; Datta and Teixeira da Silva 2006; Jain 2006; Datta 2009a, 2009b; Ntagotome and Dgi 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 induced mutations, 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 choose the one 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 mutagen methods has been ‘Faraday’ using X-ray in flowers and chlorophyll variegation in leaves can be easily detected. Heterozygous nature of many of the cultivars occur high mutation frequency.

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etities) 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 (Roebben 1959; Ehrenberg et al. 1961; Auerbach 1961; Konzak et al. 1965; Rapportor et al. 1998). Chemical mutagens have been used for 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 optimist 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, especially in 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 bulb crops, annual and perennial garden plants as well as trees and shrubs. Mutation induction techniques have been applied to the large group of ornamentals. The suitable dose of gamma rays for irradiation of stem cuttings of several mutants of diploid and triploid cultivars of chillies (1971) also studied effects of fractionated dose and different characters of ornamentals has successfully produced quite a large number of new cultivars to gamma rays (Das et al. 1990a, 1990b). The use of X-rays and gamma rays has been determined for large scale induced mutagenesis work in ornamentals (Sagawa and Mehliquist 1956, 1957, 1959; Sagawa 1957; Mehliquist 1963; Doorenbos and Karper 1975; Doorenbos 1973). The suitable dose of gamma rays for irradiation of stem cuttings of Hippeastrum 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 (Nakornhap 1965; Gupta 1966; Mukherjee and Khosshoo 1970; Desai and Abraham 1974). Induction of somatic flower colour mutation in chrysanthemum was first reported by Richter and Singleton (1995). Induced mutation frequency and other morphological alterations in chrysanthemum using medium to high radiation dose created interest on mutagenesis work in ornamentals (Sagawa and Mehliquist 1956, 1957, 1959; Sagawa 1957; Mehliquist 1963; Doorenbos and Karper 1975). 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 determined to be 1.5-2.5 Krad for small flowered chrysanthemum (Datta 1990a, 1992a, 1992b). Radio-sensitivity 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 Constantine 1966; Love and Malone 1967). A commercialized in Dahlia variabilis using radiation (Grabowska and Mynett 1964; Broertjes and Ballego 1967, 1968; Lantin and Decourtaye 1970; Das et al. 1974, 1975, 1977, 1978; Dupe 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 Queener-Ruiz 1974). Several cultivars 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 aluminum chloride, colchicine, diethyl sulfate, formaline, glycol, methyl mesahesulfonate (MMS), dimethyl sulfate, ethyl methanesulfonate (EMS), nitroso dimethyl urea (NDMU), N-nitroso-N-ethylurethran (NEU) have been used. Several crops, ornamentals and vegetables 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 rad (Datta 1992c). Radiosensitivity of large number of Bougainvillea cultivars to gamma rays have 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 characteristics of Lithium hybrids.

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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 programmes has been proposed long back (Freisleben and Lein 1943a, 1943b; Reh and Frey 1965; Broek and Shaw 1969; Miek 1969; Walther 1975). 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 programmes 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 colchicine 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 colchicine-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; Bos 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 two varieties apparently carrying the same mutant’ etc. have been recommended (Mickle 1968, 1969; Romer and Mickle 1974; Mickle 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 (Broerjtes 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. This 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 (Broerjtes 1973), Begonia (Brown and Harney 1974; Mikkelsen and Sink 1978; Roest et al. 1981; Broerjtes 1982a), Chrysanthemum morifolium (Broerjtes (Broerjtes 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; Tangsombatvitchit 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 genetic 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 tuberosa. 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; Broerjtes 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, E1) mutagens (Nakajima 1965, 1970, 1973; Chan 1966; Dommergues et al. 1967; Streitberg 1967; Kaicker and Swamp 1972; Usenbaev and Imankulova 1974; Gupta and Datta 1982; Gupta et al. 1982; Kaicker 1982, 1986; Datta and Gupta 1984a, 1985a Kaicker and Dyhani 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).
and Roest 1976; Broer tjes et al. 1976), Gerbera jamesonii (Jerzy and Lubomski 1991), Kalanchoe (Broer tjes and Leff ring 1972; Nakornthap 1974; Shama Rao and Singh 1976; Shama Rao 1977; Karper and Pierik 1981; Van Dordrecht 1984), Pelargonium (Grunewaldt 1983), Saintpaulia (Broer tjes 1972), Streptocarpus (Broer tjes 1969, 1982b), etc. About 350 plant species belonging to various plant families were detected to develop adventitious plantlets (Broer tjes 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 M1V1. Reports are also available that mutations have been detected in M1V2, M1V3 and later vegetative generations from normal looking irradiated plants in M1V1 (Buatti 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 M1V2 and later generations. Screening for mutations should not be confined to M1V1 only, but it should be continued in M1V2 and subsequent vegetative generations. The mutated cell expresses its mutant character if it gets chance to express in M1V1. The mutated cells of the lower auxiliary buds remain in the dormant stage and express its mutant character when included during vegetative propagation in M1V2 (Datta 2001).

CHLOROPHYLL VARIEGATION

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 (Broer tjes 1966; Broer tjes and van Harten 1998) and carnation (Simard et al. 1992; Cassells 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. Broer tjes et al. (1980) were successful to develop hundreds of mutants by successive use of radiation induced mutants of chrysanthemum 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 M1V1 (Maliga 1984; Ahlowlalia 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 Janek 1976; Grunewaldt and Janek 1988), carnation (Johnson 1980; Simard et al. 1991), lobelia (Lubomski 1986, 1990; Simard et al. 1991, 1992; Cassells and Walsh 1993), chrysanthemum (Jung-Heiliger and Horn 1980; Preil et al. 1983; Hultzema et al. 1986; Dowell and Preil 1987; Hultzema et al. 1989; Preil 1990; Preil 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 muta
tion by ion beams began in 1991 (Goodhead 1993; 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
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 (McCullum 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 (McCullum 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 increased the in vitro mutagenesis and are now well standardized and most promising techniques for development of new and novel varieties (Datta and Chakrabarty 2005).

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