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Recent Advances and Achievements in Mutation Breeding of Fruit Crops: A Review

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ABSTRACT

Genetic variation is essential for crop breeding. In classical plant breeding programme, variation is generated by hybridization and selections are made from the resulting segregating generations. Induced mutagenesis can supplement hybridization or able to replace as a source of variability. Since, mutations bring about variation, they provide the ultimate basis for evolution of new forms, varieties or species. Induced and spontaneous mutations have played an important role in developing improved cultivars of various fruit crops as a supplementary method to conventional breeding. But, induced mutations also have well defined limitations in fruit breeding applications, but their possibilities may be expanded by the use of *in vitro* mutation techniques.

Key words: Gene mutation, *In vitro* mutation, Irradiation, Mutagens, Tilling.

Mutations are defined as sudden heritable changes in the genetic material of an organism and in turn in its characters that are not derived from genetic segregation or recombination. Fruit crops are highly heterozygous and mutation rate is more in heterozygous material than purelines. This is due to the fact that genetic balance decreases the tendency to mutate through external influences and in fruit crops mutation is found to be more often in occurance. In fruit crops, mutagenesis has already been used to introduce many useful traits affecting plant size, blooming time, fruit ripening, fruit colour, better quality, self-compatibility, self-thinning and resistance to pathogens (Maluszynski *et al*., 1995).

On average 6 to 7 years is required by a breeder for releasing a more reliable variety than a parent. Mutation breeding will solve this problem, because it takes no time at all to breed a variety or cultivar with better character. Fruit crop improvement programme through mutation breeding was started ninety years ago and still, many improvements are being done in the way of inducing mutations in fruit crops. Recent methodologies like CRISPR Cas9 mediated mutagenesis, *in vitro* mutagenesis and mutation by gamma rays are highlighted in this article.

Based on cause of mutation, mutations are classified into spontaneous mutation and induced mutations.

Spontaneous mutation

Spontaneous mutations occur in natural population, without any human intervention. It occurs at a very low frequency 1 in 10 lakhs. They are under the control of nature and remain continuously arising in nature automatically. They are produced by naturally occurring mutagenic agents like electric currents, atomic rays and particles, injuries, disease and insect attacks, temperature, chemicals *etc.* They are the basis for crop improvement by conventional methods. Example of spontaneous mutants in fruit crops are listed in the Table 1.

Bud mutation in Indian banana is very common due to spontaneous rearrangement of chromosomes in the somatic meristem. Moongil, Attu Nendran, Nana Nendran, Nedu Nendran, Myndoli and Velathan are the mutants of Nendran variety and Sambal Monthan, Nalla Bontha Bathees, Sambrani Monthan, Pidi Montha and Thellatti Bontha are sports of cv. Monthan (Kumar, 2015).

Malladi and Hirst (2010) studied the fruit characteristics of apple cultivar Gala and its mutant Grand Gala and found that the mutant is 15 per cent larger in diameter and 38 per cent heavier than Gala fruits, together with highest TSS and lowest seed count.

Zhang and Dai (2011) reported that standard apple tree mutant derived from the crosses of Fuji × Telamon resulted in a columnar apple mutant with larger and darker green leaves, short and strong internode, higher leaf area index, higher spur: mature branch ratio (73.5 per cent), per cent of short-shoots (68.8), chlorophyll A and B content (1.878 and 0.771mg g^{-1}) than standard apple.

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In fruit crops spontaneous bud mutation are more common called as bud sports. Bud sports are infrequent changes in phenotype affecting shoots of woody perennials but the molecular basis of these mutations has rarely been identified. Among the bud sports, most common and wide spread mutations are colour alterations in red or purple anthocyanin content of fruits. Walker *et al*. (2006) documented bud sport of the wine grape Cabernet Sauvignon (*Vitis vinifera* L.) having bronze coloured berries and named as Malian. The colour mutant Malian lacks anthocyanin in the subepidermal cells compared to the red/ black berried Cabernet Sauvignon in which both the epidermis and several subepidermal cell layers contain anthocyanin.

Sharkawy *et al*. (2015) analysed apple anthocyanin deficient yellow skin somatic mutant Blondee and its red skin parent Gala and reported that anthocyanins gradually reduced in Blondee during fruit maturation.

Induced mutations

Induced mutations are artificially induced by treatment with physical, chemical and biological agents and this will change only one or a few specific traits of an elite cultivar and can contribute to fruit improvement without upsetting neither the requirements of the fruit industry nor the consumers (Lamo *et al*., 2017). Examples of induced mutants in fruit crops are listed in Table 2.

Application of 25 and 50 Gy irradiation dose respectively on sweet cherry variety ‘0900 Ziraat’ resulted in two improved varieties Aldamla and Burak (Liang, 2015). Main improved characters of Aldamla are compact growth habit (70-80%), increased fruit quality and long petioles, whereas Burak exhibits high yield, increased fruit quality and big size as improved traits.

Mutagens

A mutagen is a physical, chemical or biological agent that changes the genetic material (DNA) of an organism and thus increases the frequency of mutations above the natural incidence. Table 3 and 4 depicts the various physical and chemical mutagens used in mutation breeding programmes (Singh, 2015).

Biological mutagens used in mutation breeding

DNA elements that are able to insert at random within chromosomes are used as biological mutagens. T-DNA from *Agrobacterium tumefaciens* and transposons are the biological mutagens used and use of biological mutagens is known as insertional mutagenesis. Advantage of insertional mutagenesis over traditional form of mutagenesis is that the interrupted gene becomes tagged with the insertion element; hence the method is termed as Signature tagged mutagenesis.

Mechanism of action of mutagens

Mechanism of action of radiation

Chemical effects of radiation have direct and indirect effects. In direct effect, energy is transferred to a molecule directly by the radiation. Indirect effect is mediated by free radical formation and subsequent biochemical events are caused by reaction of highly reactive free radicals with biological molecules.

Mechanism of action of chemical mutagens

Mutagenic changes results from changes in hydrogen bonding property of bases or from mistakes in base pairing during DNA replication cause mutation by chemical agents. Inactivating alterations include removal of bases, dimer formation, cross linking of two DNA strands and single or

Table 1: Example of spontaneous mutants in fruit crops.

Crop	Original variety	Mutant cultivar	Nature of mutation and traits	Reference
Mango	Rosado de Ica	Rosica	High yielding, early ripening and regular bearing	Medina, 1977
	Haden	David Haden	Larger than Haden and matures early	Young and Ledin, 1954
Banana	Gros michel	Highgate	Sports	Daniells *et al*., 1995
	Poovan	Motta Poovan	Semi dwarf sports	Daniells *et al*., 1995
Grapefruit	Foster	Hudson	Bud sports, deep red flesh	Soost and Cameron, 1975
Pear	Clapps Favourite	Starkrimsom	Bud sports	Lamo *et al*., 2017
Mandarin	Owari Pongan	Clausellina Pongan 86-1	Bud sports	Lamo *et al*., 2017
Navel orange	Bahia	Baianinha	Limb sports	Lamo *et al*., 2017
	Washington	Navelina,Navelate, Marrs, Leng, Autumn Gold, Powell Summer, Winter Red	Limb sports	Lamo *et al*., 2017
Pomegranate	Manaozi	Hongmanaozi	-	Zhao *et al*., 2007
	Mantianhong	Taihanghong	-	Zhao, 2007
	Yushizi	Hongyushizi	-	Zhu *et al*., 2005
Almond	Nonpareil	Tardy Nonpareil	Bud sport, late flowering	Grasselly, 1978

Table 2: Examples of induced mutants in fruit crops.

Common name	Mutant variety	Parent variety	Year of release	Mutagen	Main character induced
Papaya	Pusa Nanha	Pusa 1-15	1986	Gamma rays	Shortness
Grape	Fikreti	-	1986	Gamma rays	Earliness
Indian jujube	Ma Hong	-	1986	MNH	Fruit morphology
	Dao Tien	-	1986	MNH	Earliness
Lemon	Eureka 22 INTA	Frost Eureka	1987	X-rays	Fruit set
Mandarin	Hongju 418	Dahongpaohongju (branch)	1983	Gamma rays	Seedless
	Hongju 420	Dahongpao (branch)	1986	Gamma rays	Seed number
	Xuegan 9-12-1	Xuegan (branch)	1983	Gamma rays	Seedless
Grape fruit	Rio Red	-	1984	Thermal neutrons	Fruit colour
Mulberry	Fusang 10	-	1980	Gamma rays	Internode length
	Fuzaofeng	Yu 151 (branch)	1992	Gamma rays	Earliness
	Shigu 11-6	Husang 32	1995	Gamma rays	Yield
Loquat	Shiro-mogi	Mogi	1981	Gamma rays	Fruit size
Japanese pear	Gold Nijisseiki	Nijisseiki	1993	Gamma rays	Disease resistance
	Kotobuki Shinsui	Shinsui	1996	Gamma rays	Disease resistance
Almond	Supernova	Fascionello	1987	Gamma rays	Self-compatibile
	Golden Haidegg	Golden Delicious	1986	Gamma rays	Fruit size
	Senbatsu-Fuji-2-Kei	Fuji	1985	Gamma rays	Fruit colour
Peach	Mangnif 135	-	1986	Gamma rays	Fruit size
	Plovdiv 6	-	1981	Gamma rays	Yield
Plum	Spurdente -Ferco	-	1988	Gamma rays	Earliness
Orange	Valencia 2 INTA	-	1987	Gamma rays	Fruit set
Olive	Briscola	-	1982	Gamma rays	
Raspberry	Kolokol chik	-	1991	ENH	Disease resistance
Sweet cherry	Burlat C1	-	1983	Gamma rays	Compact growth
	Nero II C1	-	1983	Gamma rays	Compact growth
	Lapins	-	1983	Cross × mutant	Fruit size
	Sunburst	-	1983	Cross × mutant	Fruit size
	Ferrovia spur	-	1992	X rays	Shortness
	Sumste samba	-	2000	Mutant cross	Fruit size

(Lamo *et al.*, 2017).

Table 3: Physical mutagens used in mutation breeding.

Mutagen	Source	Characteristics
X-rays	X-ray tube	High energy radiation, highly penetrating
Gamma rays	Radioactive decay of ^{14}C, ^{60}Co	High energy radiation, highly penetrating
Beta rays	Radioactive isotopes of ^{3}H, ^{32}P and ^{35}S	High energy electrons, shallowly penetrating
Alpha rays	Fission of radioactive isotopes of heaveir elements	Very shallowly penetrating
Neutrons	Nuclear reactors, $^{23}5$ U	Uncharged particle, highly penetrating in biological tissues

Table 4: Chemical mutagens used in mutation breeding.

Mutagen group	Example	Mode of action
Alkylating agents	Sulphonates (EMS, MMS), Sulphates, Sulphur Mustards, Nitrogen mustards, Epoxides, Imines (EI)	Nucleotide substitution (Guanine alkylation): Point mutation
Acridine dyes	Acriflavine, Proflavine, Acridine orange, Acridine yellow, Ethidium bromide	Intercalating agents: Causes frameshift mutation
Base analogues	5-Bromouracil, 5-Chlorouracil	Interchange with basepairs
Others	Nitrous acid, Hydroxyl amine, Sodium azide	

double strand breaks which prevent DNA replication across altered site and include chromosome breaks and chromosome mutations.

Mutation breeding

When mutations are induced for crop improvement, the entire operation of induction and isolation of mutants is termed mutation breeding.

Any material used for propagation can be used for mutation treatment. Seeds, pollen grains, buds or cuttings or complete plant can be used for mutagenesis. But,non-uniform penetration and distribution of mutagenic agent can leads to development of chimera. There are three types of chimera; mericlinal, periclinal and sectorial chimera. In mericlinal chimera, mutation occurs in one layer along the side of the apex. Due to its position, the cell division products of those mutated cells occur as a layer on only one side of the plant and mericlinal chimeras are not stable. In periclinal chimera, mutation occurs in one (or more) layer at the top of the apex. Due to its position, the cell division products of the mutated cells spread and cover the entire layer of the apex and entire layer is mutated. These are stable to very stable. In sectorial chimera, mutation occurs in multiple layers at the top of the apex. Due to its position, the cell division products of the mutated cells give rise to a section of mutated cells and entire section of plant is mutated. Sectorial chimeras are stable to very stable.

Mutation breeding is applied in fruit crops to increase genetic variability, to improve quantitative characters, for breaking up of close linkages, induction of male sterility, production of haploids and for overcoming self-incompatibility.

Changes in induced mutants

The changes seen in induced mutants include change in biometric, physiological and biochemical parameters. Changes in leaf area (biometric parameter), photosynthesis, hormones (physiological parameter) proline, protein, secondary metabolites and antioxidant enzymes (biochemical parameter) are noticed in induced mutants. Karsinah *et al*., (2012) reported reduced leaf area in mango with increasing gamma irradiation dosages. Alteration in photosynthetic pigments have been reported by Ling *et al*. (2008) in irradiated plantlets of sweet orange. Mutational breeding by gamma irradiation reduced average fruit seed number by 70 to 92 per cent in mandarin (Goldenberg *et al*., 2014). Citrus plantlets irradiated at high doses (30, 40 and 50 Gy) displayed a higher total soluble protein content as compared to their non-irradiated plantlets (Ling *et al*., 2008).

Ionizing radiation induces physiological changes which activate stress reaction mechanism initiating proline accumulation in plants. High X-ray dose induces enhanced proline accumulation in date palm (Al-Enezi and Al-Khayri, 2012). Phenolic metabolites constitutes the most diverse range of secondary metabolites found in plants and includes phenylpropanoids (cinnamic, coumaric, caffeic and ferulic acids) and its derivatives such as polyphenolics, namely flavonoids, anthocyanins and tannins. Flavonoids also serve as reactive oxygen species, scavenging secondary metabolites that protect the plant against oxidative stresses. A branch of *Citrus unshiu* cv. miyagawa derived from 120 Gy gamma irradiation produced fruit peel and pulp with high contents of total flavonoid pigments (Kim, 2013). Peroxidase, among antioxidant enzymes plays an important role of hydrogen peroxide detoxification in cells, thereby protecting cellular components such as proteins and lipids against oxidation. Increase in gamma irradiation dosages corresponds to an increase in specific activity of peroxidase enzymes (Ling *et al*., 2008).

Effects of gamma irradiation (physical mutagen) in fruit crops

Gamma irradiation in fruit crops helped in production of seedless fruits, imparting dwarfness, improving seed germination, yield parameters, fruit quality, keeping quality, attaining disease resistance, developing ornamental plants and production of haploid plants.

Effect of gamma irradiation on seedlessness in fruit crops

PAU Kinnow-1 is the seedless mutant of Kinnow obtained by irradiating budwoods of Kinnow with 30 Gy. The seed count was reduced from 20.7 to 3.40 (Rattanpal *et al*., 2015). Pummelo variety ‘Pamelo Nambangan’ budwood when exposed to 20 Gy irradiation developed a mutant with improved character on the number of seeds and was released as ‘Pamindo Agrihorti’ (Mariana *et al*., 2018). Gamma radiation induced chromosomal aberrations caused a significantly higher abortion rate (86.21%) of megagametophyte during megasporogenesis, which contributes to seedlessness in fruits (Huang *et al*., 2017).

Effect of gamma irradiation on dwarfing in fruit crops

Ram and Srivastava (1984) exposed dry seeds of papaya cv. Pusa 1-15 with 15 Krad of gamma rays and obtained one dwarf compact mutant in M_3 generation and its homozygosity was achieved in M_6 generation by repeated full sib mating.

Influence of gamma irradiation on plant height in papaya varieties 10 months after planting was studied by Husselman *et al*. (2016) and found that varieties Bh 65, Sunrise Solo and Tainung produced dwarf plants at 80 Gy with 74.50, 82.17 and 126 cm height respectively against 99.63, 166.25 and 136.40 cm for control.

Germinated seeds of papaya variety Dai Loan Tim were irradiated with gamma rays ranging from 10 to 60 Gy and from the progenies, four mutant plants, which were particularly outstanding in vigour with medium dwarf stature was identified by Hang and Chau (2010). The four mutant dwarf selections (M 064, M 070, M 160 and M 313) were found to bear at a height of 58, 56, 60 and 58 cm from the ground and after 120 to 125 days of planting attained a total height of 160, 145, 162 and 164 cm, respectively. Whereas, the parent plant Dai Loan Tim bear flowers at a height of 90 cm and attained a total height of 205 cm.

Effect of gamma irradiation on seed germination in fruit crops

Hafiz *et al*. (2005) irradiated mango stones of the cultivar Dashehri, sourced from cobalt-60 and found that germination percentage was significantly highest (87.5) with 2.5 Kr.

Husselman *et al*. (2016) identified 50 Gy and 80 Gy as suitable irradiation dosages for higher germination percentage in varieties V3 and Tainung repectively.

The stimulatory effects of gamma rays on germination may be attributed to the activation of RNA synthesis or protein synthesis which occurred during the early stage of germination (Kuzin *et al*., 1976). The inability of seeds to germinate at higher doses of gamma rays has been attributed to numerous histological and cytological changes, disruption and disorganisation of seed layer that is directly proportional to the intensity of exposure to gamma rays, impaired mitosis or virtual elimination of cell division in the meristematic zones during germination (Lokesha *et al*., 1992). Also the inhibition of seed germination and seedling growth exerted by irradiation has often been ascribed to the formation of free radicals in irradiated seeds (Kovacs and Keresztes, 2002).

Impact of gamma irradiation on yield parameters in fruit crops

Zamir *et al*. (2009) reported that 0.15 KGy of gamma rays resulted in highest number of fruits in guava. Brunner and Keppl (1991) found lesser fruit russetting percentage and higher total yield in mutant 'Golden Haidegg' compared to the parent Golden delicious apple.

Effect of gamma irradiation on fruit quality

Evaluation of citrus fruit peel colour revealed that gamma irradiation mutagenesis of bud wood material significantly enhanced the colour change of the early season mandarin varieties Kedem and irradiated Michal, as compared with that of the control (Goldenberg *et al*., 2014).

Effects of gamma-irradiation on TSS and Vitamin C of mandarin varieties and its mutants were studied by Goldenberg *et al*. (2014). Kedem (mutant of Rishon) and Mor (mutant of Murcott) recorded highest TSS (11.12 and 15.05% respectively) and vitamin C content (44.94 and 31.20 mg 100 mL^{-1} respectively) compared to Rishon (10.23% and 35.84 mg 100 mL^{-1}) and Murcott (13.10% and 30.64 mg 100 mL^{-1}). Fruit flavour of three irradiated varieties Kedem, Vardit and irradiated King was found significantly preferable over the parents Rishon, Vered and King on evaluation using trained sensory panel (Goldenberg *et al*., 2014).

Effect of gamma irradiation on disease resistance in fruit crops

Black spot disease (*Alternaria alternata*) resistant japanese pear mutant 'Gold Nijisseiki' was developed by chronic irradiation of Nijisseiki with 616.2 Gy (Yoshioka *et al*., 1999).

Effect of gamma irradiation on developing ornamental fruit plants

Mutants with variegated fruit and enlarged base was reported in Murcott mandarin and Fino 49 lemon respectively by Montanola *et al*. (2015). A new lemon genotype with potential use for ornamental purposes having attractive appearance with vertical brown lines and brown point heaps was reported by Uzun *et al*. (2015) by exposing 'Kutdiken' lemon cultivar budwood to gamma irradiation.

Effect of gamma irradiation on production of haploid plants

Haploids are favoured material for mutation studies as both recessive and dominant mutation can be identified and subsequent chromosome doubling results in a homozygous genotype. Induction of parthenogenesis by pollination with gamma irradiated pollens is an efficient method to obtain haploid plants. Cross pollination of 'Hirado Buntan' pummelo with irradiated pollens of trifoliate orange and 'Tongshui 72-1 Jincheng' sweet orange produced two haploid plants (Wang *et al*., 2016).

Effects of ethyl methane sulphonate (chemical mutagen) in fruit crops

Papaya seeds of cv. Pusa Dwarf were treated with different doses (0.25%, 0.50%, 0.75% and 1.00%) of Ethyl Methane Sulphonate (EMS) to observe the influence of treatment on fruit quality of papaya (Kumar *et al*., 2017). Fruits obtained from the seeds treated with 0.50 per cent EMS had significantly maximum fruit length, fruit girth, fruit weight, pulp thickness, TSS, sugar, fat, ash, carbohydrate, protein, carotene and minimum central cavity and moisture content. The stimulatory effect of EMS at a lower dose is due to the fact that mutagens at lower concentration stimulate enzyme and growth hormone responsible for growth, yield and fruit quality, while, higher concentration of mutagens had inhibitory effect on fruit quality. Thus, fruit yield and quality of genetically modified papaya can be improved significantly through induced mutagenesis by ethyl methane sulphonate.

Effects of T- DNA (biological mutagen) insertional mutagenesis in fruit crops

Insertional mutant collections are essential tools in plant genomic research and functional genomics. The concept is based on random insertion of foreign DNA into promoters or gene encoding regions in order to disrupt the function of native genes and thereby gain an understanding of their roles. Phenotype of a plant carrying such an insertion is expected to differ from that of wild type plants only by changes that are associated with the functions of a single gene disruption. Insertional mutagenesis in the diploid strawberry (*Fragaria vesca*) was studied by Veilleux *et al*. (2011). They observed morphological mutants like petalless flower, reduced flower petal, golden leaved plant and dwarf plant with mottled leaves of *Fragaria vesca* after transformation with pCAMBIA vector 1302.

Limitations of mutation breeding

- Frequency of desirable mutation is very low. Hence, mutation breeding involves considerable time, labour and other resources.
- Since the breeder has to screen large populations, selection of mutants is cumbersome.
- Desirable mutations are commonly associated with undesirable side effects due to other mutations, chromosomal aberrations *etc*. Therefore, mutant lines often have to be backcrossed to the respective parent varieties to remove these defects. This increases time and resources needed for developing mutant varieties.
- Most of the mutations are recessive. Detection of recessive mutations is almost impossible in clonal crops and is difficult in polyploidy species.
- There may be problems in registration of mutant variety since it may be difficult to convincingly demonstrate the new variety to be distinct from the parent variety.

In vitro mutagenesis

In vitro mutagenesis is a method combining mutation breeding with tissue culture and it has proved more effective rather than the conventional breeding and increases the efficiency of mutagenic treatments for variation induction (Predieri, 2001). More recently, the induction of mutations in vegetatively propagated plants are becoming more efficient as scientists take advantage of totipotency using single cells and other forms of in vitro cultured plant tissues. This method gives less risk of obtaining chimeric plants and a higher probability for mutated cells to express the mutation in the phenotype (D'Amato, 1977).

In vitro mutagenesis in banana

Breeding program of edible bananas is hampered by high sterility and very limited amounts of seeds (slow propagation rate). Few diploid banana clones produce viable pollen. Somatic embryogenic cell suspension is highly suitable for mutation induction and genetic transformation in banana. Mutation induction is achieved by exposing Embryogenic Cell Suspension (ECS) to gamma irradiation (Lopez *et al*., 2017). Mutant selection occurs both during the acclimatization phase and under field conditions. The main advantage of using the ECS for *in vitro* mutagenesis treatment is the instant production of non-chimeric populations or the quick dissociation of the chimeric sectors if they are found.

Achievements of *in vitro* mutagenesis in fruit crops

Several varieties have developed by *in vitro* mutagenesis technique in fruit crops. Those are listed in the Table 5.

Effect of *in vitro* mutagenesis on root formation

Bhat *et al*. (2015) conducted experiment to create variability through induced mutations in different explants (runner tip, shoot tip, leaf disc (abaxial and adaxial)) of strawberry cv. Camarosa. EMS dose of 0.1 per cent applied for 1.5 hr on runner tip explants was found best among all treatments in terms of *in vitro* root growth parameters recorded *viz*., initiate early roots (45.3 days), maximum number of roots (32.5) and maximum length of roots (55.5 mm).

Effect of *in vitro* mutagenesis on reproductive growth

Different explants (runner tip, shoot tip, leaf disc (abaxial and adaxial) of strawberry cv. Camarosa were subjected to different EMS concentrations (0.1%, 0.2%, 0.3% and 0.4%) to study the effect of induced mutation on reproductive growth. The runner tip explants treated with EMS concentration 0.1 per cent for 1.5 hr duration took minimum days to bear first flower (25.5) and maximum number of flowers per plant (25.5) (Bhat *et al*., 2017a).

Effect of *in vitro* mutagenesis on yield parameters in fruit crops

Effect of EMS induced mutation in strawberry cv. Camarosa for yield parameters was studied by Bhat *et al*. (2017b). Runner tip explants treated with EMS concentration 0.1 per cent for 1.5 hr duration gave maximum number of fruits per plant (20.5), yield per plant (0.35 kg) and fruit size (47.1 mm × 41.5 mm) and take minimum days to fruit maturity (20.2 days) with dark red, conical shaped fruit.

Table 5: Mutants developed by *in vitro* mutagenesis.

Crop species	Treated material	Mutagen and dose	Plant regeneration route	Selected mutants/lines
Banana (*Musa spp.*)	Shoot tips	γ-rays (60 Gy)	Direct regeneration	Mutant Novaria; earliness
Banana *var*. Lakatan	Shoot tips	γ-rays (40 Gy)	Direct regeneration	Height reduction, larger fruit size
Banana *var*. Latundan	Shoot tips	γ-rays (25 Gy)	Direct regeneration	Mutant Klue Hom Thong KU1
Pineapple *var*. Queen	Crowns	γ-rays	Axillary bud regeneration	Lines with reduced spines
Pear	*In vitro* shoots	*In vitro* shoots	Microcuttings from shoot	Mutants free from russeting, small tree, short internodes

(Ahloowalia, 1995).

Effect of *in vitro* mutagenesis for disease resistance in fruit crops

Shoot apices of *in vitro* grown cultures of banana *Musa* spp., AAA Group cv. Highgate were treated with various concentrations of chemical mutagens sodium azide, diethyl sulphate and ethyl methane sulphonate to evaluate their effectiveness in inducing mutations. Twelve weeks after inoculation with Fusarium oxysporum f. sp. cubense, 4.6, 1.9 and 6.1 per cent of plants regenerated after sodium azide, diethyl sulphate and ethyl methane sulphonate mutagenesis respectively had less than 10 per cent vascular invasion of their corms with no external symptoms of disease (Bhagwat and Duncan, 1998).

Effect of *in vitro* mutagenesis for abiotic stress resistance in fruit crops

Development of salt tolerant strawberry by irradiation of callus with 20-50 Gy gamma rays and subsequent treatment with 200 Mm NaCl was done by Dziadczyk *et al*. (2003).

Effect of *in vitro* mutagenesis for developing tetraploids in fruit crops

Shoot tips of self incompatible olive diploid Leccino, when treated with gamma rays produced tetraploid Leccino with self fertile character (Rugini *et al*., 2016).

Effect of *in vitro* mutagenesis on dwarfing in fruit crops

Apical and axillary buds of *in vitro* cultures of cherry rootstock ‘F 12/1’ were given treatment with colchicine 0.1 per cent for 48 hours and gamma irradiation with 30 Gy (Hedtrich, 1990) and found that colchicine was superior to gamma irradiation in producing dwarfing in cherry rootstock ‘F 12/1’.

Effect of somaclonal variation in producing mutations

Regeneration of plants from undifferentiated cells in culture is frequently a source of variability, known as somaclonal variation.

Detached leaf bioassay of peach [*Prunus persica* (L.) Batsch] somaclone 122-1, for resistance to several virulent strains of *Xanthomonas campestris* pv. *pruni*, was done by Hammerschlag (2000). Somaclone 122-1 was significantly more resistant to most strains of *X. campestris* pv. *pruni* than the parent ‘Redhaven’.

Crispr Cas9 mediated mutagenesis

CRISPR Cas9 is a special genome editing technology in which breeder can precisely manipulate the gene by removing, adding or altering sections of the DNA sequence. Achievements of Crispr Cas9 mediated mutagenesis in fruit crops are many. Wang *et al*. (2018) reported that in grapevine, CRISPR Cas9 mediated mutagenesis resulted in knockout of transcription factor VvWRKY52 and made it resistant to *Botrytis cinerea*. Aroma biosynthesis in peach fruit was reported to be enhanced by editing the catalyzing factor PpAAT1, using CRISPR Cas9 (Peng *et al*., 2020). Knocking out of genes using CRISPR Cas 9, developed β-carotene enriched Cavendish banana (Kaur *et al*., 2020).

Detection of mutation

Traditionally, mutants were mostly selected by observing phenotypes of individual plants in mutated populations treated with chemical or physical mutagens. But nowadays, molecular markers are widely used to differentiate the genetic differences between the mutant and the mother plants through characterizing the variations at DNA level. Random Amplified Polymorphic DNA (RAPD), cDNA-amplified fragment length polymorphism (AFLP), single-strand conformation polymorphism (SSCP), microarray, targeting induced local lesions in genome (TILLING) and high-resolution melt (HRM) analysis allow rapid and in depth analysis of mutational variations (Celik and Atak, 2017).

CONCLUSION

Mutation is an important breeding tool for creating variability in fruit crops. It provides an opportunity for the improvement of traits like dwarf plant, earliness, tolerance and resistance to various diseases and pests within short period of time. Mutant identification or selection at the genotypic level, using new technologies, has changed the way mutations are now used in genetics and breeding in fruit crops. *In vitro* culture combined with induced mutation had been proven to speed up the breeding program to produce genetic variations or for multiplication. It also helps in development of commercial varieties for achieving the target of nutritional security.

Conflict of interest: None.

REFERENCES

Ahloowalia, B.S. (1995). *In vitro* Mutagenesis for the Improvement of Vegetatively Propagated Plants. [(Eds) Jain, S.M. and Brar, D.S.] Proceedings of Joint FAO/IAEA International Symposium. 28-30 January 1995, Vienna, pp. 531-541.

Al-Enezi, N.A. and Al-Khayri, J.M. (2012). Effect of X-irradiation on proline accumulation, growth and water content of date palm (*Phoenix dactylifera* L.) seedlings. Journal of Biological Science. 12(3): 146-153.

Bhat, S., Sharma, S. and Sharma, V.K. (2017a). Influence of *in vitro* induced mutation on reproductive growth of camarosa strawberry. Agriculture Update 12(3): 676-680.

Bhat, S., Sharma, S. and Sharma, V.K. (2017b). Effect of EMS induced morphological mutation in strawberry. Journal of Hill Agriculture. 8(1): 50-55.

Bhat, S., Sharma, S., Sharma, V.K., Pratap, P. and Kajla, S. (2015). Effect of concentration and application duration of EMS on *in vitro* root formation of camarosa strawberry (*Fragaria* × *Ananassa*). The Bioscan. 10(1): 187-191.

Brunner, H. and Keppl, H. (1991). Radiation induced apple mutants of improved commercial value. Plant Mutation Breeding for Crop Improvement. 1: 547-552.

Bhagwat, B. and Duncan, E.J. (1998). Mutation breeding of banana cv. Highgate (*Musa* spp. AAA Group) for tolerance to *Fusarium oxysporum* f. sp. *cubense* using chemical mutagens. Scientia Horticulturae. 7: 11-22.

Celik, O. and Atak, C. (2017). Applications of ionizing radiation in mutation breeding. Genetics and Molecular Research. 14(1): 1324-1337.

Daniells, J., Davis, D., Peterson, R. and Pegg, K. (1995). Goldfinger: Not as resistant to sigatoka or yellow sigatoka as first thought. Infomusa. 4(1): 6.

D'Amato, F. (1977). Cytogenetics differentiation in tissue and cell cultures. Applied and Fundamental Aspects of Plant Cell Tissue and Organ Culture. 343-357.

Dziadczyk, P., Bolibok, H., Tyrka, M. and Hortynski, J.A. (2003). *In vitro* selection of strawberry (*Frageria ananassa* Duch.) clones tolerant to salt stress. Euphytica. 132(1): 49-55.

Goldenberg, L., Yaniv, Y., Porat, R. and Carmi, N. (2014). Effects of gamma-irradiation mutagenesis for induction of seedl essness on the quality of mandarin fruit. Food and Nutrition Science. 5(10): 943-952.

Grasselly, C. (1978). Observations on the use of a late flowering almond mutant in a hybridization program. Ann d'Ame´ lioration des Plantes. 28: 685-695.

Hafiz, I.A, Anwar, N., Abbasi, N.A. and Asi, A.A (2005). Effect of various doses of gamma radiation on the seed germination and seedling growth of mango. Sarhad Journal of Agriculture. 21(4): 563-567.

Hammerschlag, F.A. (2000). Resistant responses of peach somaclone 122-1 to *Xanthomonas campestris* pv. *pruni* and to *Pseudomonas syringae* pv. *syringae*. Hort Science. 35(1): 141-143.

Hang, N.N.T. and Chau, N.M. (2010). Radiation induced mutation for improving papaya variety in Vietnam. Acta Horticulturae. 851: 77-80.

Hedtrich, R.T. (1990). Induction of dwarf F-12/1 cherry rootstocks by *in vitro* mutagenesis. Acta Horticulturae. 280: 367-374.

Huang, J.H., Wen, S.X., Zhang, Y.F., Zhong, Q.Z., Yang, L. and Chen, L.S. (2017). Abnormal megagametogenesis results in seedlessness of a polyembryonic 'Meiguicheng'orange (*Citrus sinensis*) mutant created with gamma-rays. Scientia Horticulturae. 217: 73-83.

Husselman, J.H., Daneel, M.S., Sippel, A.D. and Severn Ellis, A.A. (2016). Mutation breeding as an effective tool for papaya improvement in South Africa. Acta Horticulturae. 1111: 71-78.

Karsinah, N.L.P., Indriyani. and Sukartini. (2012). The effect of gamma irradiation on the growth of mango grafted material. Journal of Agriculture and Biological Science. 7(10): 840-844.

Kaur, N., Alok, A., Shivania, Kumara, P., Kaura, N., Awasthia, P., Chaturvedi, S., Pandeya, P. and Pandeya, A. (2020). CRISPR/Cas9 directed editing of lycopene epsilon-cyclase modulates metabolic flux for β-carotene biosynthesis in banana fruit. Metabolic Engineering. 59: 76-86.

Kim, M.Y. (2013). Analysis of bioactive compounds in gamma irradiation induced citrus mutants. Life Science Journal. 10(10): 210-214.

Kovacs, E. and Keresztes, A. (2002). Effect of gamma and UV-B/C radiation on plant cells. Micron. 33(2): 199-210.

Kumar, M., Kumar, M. and Chaudhary, V. (2017). Effect of seed treatment by ethyl methane sulphonate (EMS) on fruit quality of papaya (*Carica papaya* L.) cv. Pusa Dwarf. International Journal of Applied Chemistry. 13(1): 145-150.

Kumar, N. (2015). Breeding of Horticultural Crops. Principles and Practices, New India Publishing Agency.

Kuzin, A.M., Vagabova, M.E. and Revin, A.F. (1976). Molecular mechanisms of the stimulating action of ionizing radiation on seeds. Radiobiology. 16: 259-261.

Lamo, K., Bhat, D.J., Kour, K. and Solanki, S.P.S. (2017). Mutation studies in fruit crops: A review. International Journal of Current Microbiology and Applied Science. 6(12): 3620-3633.

Liang, Q. (2015). Sweet cherry mutant varieties released in turkey. Plant Genetic Resources. 10(2): 101-107.

Ling, A.P.K., Chia, J.Y., Hussein, S. and Harun, A.R. (2008). Physiological responses of *Citrus sinensis* to gamma irradiation. World Applied Science Journal. 5(1): 12-19.

Lokesha, R., Vasudeva, R., Shashidhar, H.E. and Reddy, A.N.Y. (1992). Radio-sensitivity of bambusa arundinacea to gamma rays. Journal of Tropical Forest Science. 6(4): 444-450.

Lopez, J., Rayas, A., Santos, A., Medero, V., Beovides, Y. and Basail, M. (2017). Mutation Induction Using Gamma Irradiation and Embryogenic Cell Suspensions in Plantain (*Musa* spp.). In: Biotechnologies for Plant Mutation Breeding, Springer Nature, Switzerland. [Joanna, J.C., Thomas, H. T., Jochen, K. and Bradley, J. T. (eds.)]. pp. 55-71.

Malladi, A. and Hirst, P.M. (2010). Increase in fruit size of a spontaneous mutant of Gala apple (*Malus× domestica* Borkh.) is facilitated by altered cell production and enhanced cell size. Journal of Experiemental Botany. 61(11): 3003-3013.

Maluszynski, M., Ahloowalia, B.S. and Sigurbjornsson, B. (1995). Application of *in vivo* and *in vitro* mutation techniques for crop improvement. Euphytica. 85: 303-315.

Mariana, B.D., Arisah, H., Yenni and Selvawajayanti, M. (2018). Seedless fruit pummelo induced by gamma ray irradiation: Fruit morphological characters and stability evaluation. Biodiversitas. 19: 706-711.

Medina, J.P. (1977). Rosica a new mango variety selected in Ica. Peru. Fruit variety Journal. 31: 88-9.

Montanola, M.J., Galaz, A., Gambardella, M. and Martiz, J. (2015). New low seeded mandarin (*Citrus reticulata*) and lemon (*C. limon*) selections obtained by gamma irradiation. Acta Horticulturae. 1065: 543-548.

Peng, B., Xu, J., Cai, Z. and Zhang, B. (2020). Different roles of the five alcohol acyltransferases in peach fruit aroma development. Journal of the American Society for Horticultural Science. 145(6): 374-381.

Predieri, S. (2001). Mutation induction and tissue culture in improving fruits. Plant Cell Tissue Organ Culture. 64: 185-210.

Ram, M. and Srivastava, S. (1984). A note on occurrence of mutants in papaya [abstract]. In: Abstracts, National Seminar on Papaya and Papain Production; 26-27 March, 1984, Coimbatore, p.28. Abstract No.23.

Rattanpal, H.S., Sidhu, G.S. and Uppal, G.S. (2015). Development of low seeded kinnow through mutation breeding. Agriculture Research Journal. 52(2): 198-199.

Rugini, E., Silvestri, C., Ceccarelli, M., Muleo, R. and Cristofori, V. (2016). Mutagenesis and biotechnology techniques as tools for selecting new stable diploid and tetraploid olive genotypes and their dwarfing agronomical characterization. Hort Science. 19: 311-319.

Sharkawy, I., Liang, D. and Xu, K. (2015). Transcriptome analysis of an apple (*Malus* × *domestica*) yellow fruit somatic mutation identifies a gene network module highly associated with anthocyanin and epigenetic regulation. Journal of Experiemental Botany. 66(22): 7359-7376.

Singh, B.D. (2015). Plant Breeding: Principles and Methods. Kalyani publishers, New Delhi. 923p.

Soost, R.K. and Cameron, J.W. (1975). Citrus. In: Advances in Fruit Breeding, Purdue, University Press. [Janic, J. and Moore, J. N. (eds.)], West Lafayett, Indiana. pp. 507-540.

Uzun, A., Gulsen, O., Kafa, G. and Seday, U. (2015). New lemon genotype for ornamental use obtained from gamma irradiation. Acta Horticulturae. 1065: 245-247.

Veilleux, R.E., Oosumi, T., Wadl, P.A., Baxter, A.J., Holt, S.H., Ruiz-Rojas, J.J. and Shulaev, V. (2011). Insertional mutagenesis in the diploid strawberry (*Fragaria vesca*). Acta Horticulturae. 941: 49- 54.

Walker, A.R., Lee, E. and Robinson, S.P. (2006). Two new grape cultivars, bud sports of Cabernet Sauvignon bearing pale-coloured berries, are the result of deletion of two regulatory genes of the berry colour locus. Plant Molecular Biology. 62: 623- 635.

Wang, X., Tu1, M., Wang, D., Liu, J., Li1, Y., Li., Z., Wang, Y. and Wang X. (2018). CRISPR/Cas9- mediated efficient targeted mutagenesis in grape in the first generation. Plant Biotechnology Journal. 16: 844-855.

Wang, S., Lan, H., Jia, H., Xie, K., Wu, X., Chen, C. and Guo, W. (2016). Induction of parthenogenetic haploid plants using gamma irradiated pollens in 'Hirado Buntan' pummelo [*Citrus grandis* (L.) Osbeck]. Scientia Horticulturae. 207: 233-239.

Yoshioka, T., Masuda, T., Kotobuki, K., Sanada, T. and Ito, Y. (1999). Gamma Ray Induced Mutation Breeding in Fruit Trees, JARQ. 33: 227- 234.

Young, T.W. and Ledin, R.B. (1954). Mango breeding. Proccedings of Florida State of Horticulture Society. 67: 241-244.

Zamir, R., Ali, N., Shah, S.T., Mohammad, T. and Ahmad, J. (2009). Guava (*Psidium guajava* L.) improvement using *in vivo* and *in vitro* induced mutagenesis. Induced Mutation in Tropical Fruit Trees. 101-112.

Zhang, Y.G. and Dai, H.Y. (2011). Comparison of photosynthetic and morphological characteristics and microstructure of roots and shoots, between columnar apple and standard apple trees of hybrid seedlings. Phyton-Revista Internacional de Botanica Experimental. 80: 119-125.

Zhao, C.L. (2007). Breeding of early pomegranate cultivar "Taihanghong". China Fruits. 3: 5-6.

Zhao, G.R., Zhu, L.W., Zhang, S.M., Jia, B. and Li, S.W. (2007). A new soft-seeded pomegranate variety, Hongmanaozi. Acta Horticulturae Sinica. 34(1): 260-260.

Zhu, L.W., Zhang, S.M., Gong, X.M., Jia, B., Li, S.W. and Li, Y. (2005). A new soft seeded pomegranate variety-Hongyushizi. Acta Horticulturae Sinica. 32(5): 965.