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Mutation Studies in Fruit Crops: A Review

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A B S T R A C T

Mutation is sudden heritable changes in the DNA sequence that are not derived from genetic segregation or recombination. Spontaneous mutation occurs at a very low frequency 1 in 10 lacs or 10^{-6}. In fruit crops spontaneous bud mutation are more common called as bud sports. The occurrence of a large number of natural bud sports in citrus, mango, grapes etc. made the fruit breeders interested to breed through induced mutation. Since, mutations bring about variation, they provide the ultimate basis for evolution of new forms, varieties or species. Mutations may result into deletion, inversion, translocation of chromosome and nucleotide base substitutions. Mutation can be induced artificially with the help of various physical and chemical agents which are called mutagens. Most commonly used Physical and chemical mutagens are gamma rays and EMS (Ethyl Methane Sulphonate) respectively. Dose inducing 25 to 50% lethality (LD_{25}-LD_{50}) among mutated plants will result in the highest mutation rates. Perennial nature, long juvenile phase, heterozygosity, sexual incompatibilities etc in fruit crops limits their improvement through conventional breeding.

Introduction

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 (Van Harten, 1998). De Vries used the word "sudden" to differentiate between subtle changes that could be explained by the normal processes of recombination. The origins were in the variants described in taxonomic treatises of wild and cultivated plants and in spontaneous mutants (bud sports, etc) of value and interest in domestication and crop improvement. The term mutation breeding was coined to refer to the deliberate induction and development of mutant lines for crop improvement. The term has also been used in a wider sense to include the exploitation of natural as well as spontaneous mutants, and in the development of any variety possessing a known mutation from whatever source. Mutants are the individuals carrying a mutation that may be revealed using molecular means or identified by phenotypic tools. Different types of mutant can be generated using experimental mutagenesis. Mutagens these are the agent use to induce mutation. Spontaneously arising mutations are very rare and random events in terms of the time of their occurrence and the gene in which they occur. In this way mutant forms showing both large and small effects on the phenotype arise for all kinds of traits.

Many of the mutations may be deleterious making the organism less adapted to its environment and some may even be lethal. Mutagenesis can be exploited experimentally (experimental mutagenesis) by physical, chemical and biological means. The fruit industry relies on a limited number of clonally propagated cultivars established on recognized fruit quality parameters and consumer familiarity with the product, and is very reluctant to changes (Janick, 1992). This limits the use of cross-breeding in fruits, as fruit cultivars are generally highly heterozygous, and progenies from cross-breeding express a large number of traits which are different from those of the parents. Other specific problems (e.g. polyploidy, incompatibility, apomixis, long juvenile period) can make obtaining useful recombinants a laborious task (Broertjes, 1977; Hansche and Beres, 1980). In contrast, induced mutations 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. In fruit crops, mutagenesis has already been used to introduce many useful traits affecting plant size, blooming time and fruit ripening, fruit color, self-compatibility, self-thinning, and resistance to pathogens (Visser *et al.,* 1971; Janick and Moore, 1975; Donini, 1982; Broertjes and Van Harten, 1988; Spiegel-Roy, 1990; Spina *et al.,* 1991; Brunner and Keppl, 1991; Masuda *et al.,* 1997; Janick and Moore, 1996; Van Harten, 1998; Sanada and Amano, 1998).

Types of mutation

Spontaneous mutation

Spontaneously arising mutations are very rare and random events in terms of the time of their occurrence and the gene in which they occur. In this way mutant forms showing both large and small effects on the phenotype arise for all kinds of traits. Many of the mutations may be deleterious making the organism less adapted to its environment and some may even be lethal. Some may be neutral in their effects and may confer no immediate advantage, but may help to generate a wide range of useful recombinant genotypes through the subsequent generations. However, on rare occasions, some cultivars can demonstrate unstable phenotypes resulting in a portion of the plant, sometimes extending to whole branches, having different characteristics. When these branches (bud sports) are vegetatively propagated by clonal techniques, the new phenotype is generally maintained leading to a new variety, often exhibiting only one phenotypic character different from the parent (Marcotrigiano 1997). Horticulturist propagates bud sports because they continue to exhibit all of the other desirable characteristics of the parent (Franks *et al.,* 2002). Bud sports are infrequent changes in phenotype affecting shoots of woody perennials but the molecular basis of these mutations has rarely been identified (Table 1).

When a mutation (plastidial, genic, chromosomal, genomic) arises in a cell within a shoot apical meristem of a bud, the mutated cell propagates mitotically and produces a mutated sector (sectorial or mericlinal chimera) (D' amato 1977). During further vegetative growth of the initial bud one or more wholly mutated buds (solid mutant or sport may be produced. Mutated buds may develop into an independent plant through various naturally occurring modes of vegetative propagation (tubers, tubercles, tuberous roots, bulbs, pseudobulbs, cormets, stolons, rhizomes, root crowns, stem points, inflorescence bulbils, adventitious embryony and apomictic seed).Obviously, sports can also be propagated through true (amphimictic) seed when the original plant reproduces

sexually. Among the bud sports, the most common and wide spread mutations are colour alterations in the red or purple anthocyanin content of fruit (Walker *et al.*, 2006). Compared with conventional hybridization breeding, bud sport is a consequence of genetic variation of somatic cells leading to the occurrence of qualitative and quantitative phenotypic alteration in plants, which can be observed in many vegetatively propagating plants including grapes (Liu *et al.*, 2007). Bud mutants have been widely exploited by vine growers to develop new cultivars of wine grapes and table grapes. Zhao *et al.*, (2014) reported an 'Early-ripening Benitaka' grape, a bud sport of the 'Benitaka' (*Vitis vinifera*) cultivar that exhibits a marked difference in sugar and anthocyanin accumulation in ripening fruit arises from a spontaneous mutation. Walker *et al.*, (2006) reported that two new grape cultivars which were bud sports of Cabernet Sauvignon namely Malian or Bronze Cabernet berries and Shalistin or White Cabernet berries. Liu *et al.*, (2007) reported a spontaneous bud mutation in sweet orange (*Citrus sinensis* L. Osbeck) 'Hong Anliu', which results in fruits with lycopene accumulation, low citric acid, and high sucrose was reported in a previous study.

Induced mutation

Agents of artificial mutations are called mutagens. They are generally grouped into two broad categories, namely chemical mutagens and physical mutagens (Mba *et al.*, 2010). Traditionally, to induce mutations in crops, planting materials are exposed to physical and chemical mutagenic agents. Mutagenesis can be performed with all types of planting materials, e.g. whole plants, usually seedlings, and *in vitro* cultured cells. Nevertheless, the most commonly used plant material is seed. Multiple forms of plant propagules, such as bulbs, tubers, corms, shoot tip, leaf, ovules, protoplasts and rhizomes. and more recently, the induction of mutations in vegetatively propagated plants is becoming more efficient as scientists take advantage of totipotency (ability of a single cell to divide and produce all of the differentiated cells in an organism to regenerate into whole plants) using single cells and other forms of *in vitro* cultured plant tissues. Whereas chemical mutagens are preferably used to induce point mutations, physical mutagens induce gross lesions, such as chromosomal abbreviation or rearrangements. It is noteworthy that the frequency and types of mutations are direct results of the dosage and rate of exposure or administration of the mutagen rather than its type Mba *et al.*, (2012). In the end, the choice of a mutagen will be based more often than not on the particular researcher's circumstances, such as safety of usage, ease of use, availability of the mutagens, effectiveness in inducing certain genetic alterations, suitable tissue, cost and available infrastructure among other factors. Tissue culture has a potential for improving effectiveness of mutation induction in several aspects. First of all, it offers a wide choice of plant material for treatment that mutation induction. In fact, structures that will give origin to plants are composed of a few or even of one cell. This means less risk of obtaining chimaeric plants and a higher probability for mutated cells to express the mutation in the phenotype (D' Amato, 1977). Tissue culture also allows for the handling of large populations for mutagenic treatment, selection, and cloning of selected variants. It also offers the possibility to rapidly execute the propagation cycles of subculture aimed to separate mutated from non-mutated sectors (dissolving a chimera to obtain homo-histone) (Ahloowalia, 1998). Despite these prospects and a number of research studies, only two fruit cultivars derived from *in vitro* mutation induction have been released to date i.e.

cultivars of banana (*Musa acuminata* Colla) 'Klue Hom Thong KU1' and 'Novaria'. These poor results suggested that mutagenesis in combination with tissue culture is either ineffective or has yet to be exploited in fruit crops. The availability of tissue culture protocols for plant micropropagation and regeneration from cell and tissues for nearly all of the fruits commercially most important (George, 1993; Herman, 2000) calls for a wider use of *in vitro* mutation induction. The fruit industry relies on a limited number of clonally propagated cultivars established on recognized fruit quality parameters and consumer familiarity with the product, and is very reluctant to changes (Janick, 1992; Mehlenbacher, 1995). This limits the use of cross-breeding in fruits, as fruit cultivars are generally highly heterozygous, and progenies from cross-breeding express a large number of traits which are different from those of the parents. Other specific problems (e.g. polyploidy, incompatibility, apomixis, long juvenile period) can make obtaining useful recombinants a laborious task (Broertjes, 1977; Hansche and Beres, 1980). In contrast, induced mutations 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.

Physical mutagens

In the past 80 years, physical mutagens, mostly ionizing radiations, have been used widely for inducing hereditary aberrations and more than 70% of mutant varieties were developed using physical mutagenesis (Mba *et al.*, 2012). Radiation is defined as energy travelling through a distance in the form of waves or particles. These are relatively high-energy levels of electromagnetic (EM) spectrum that are capable of dislodging electrons from the nuclear orbits of the atoms that they impact upon. The impacted atoms become ions therefore, the term ionizing radiation was coined. These ionizing components of the electromagnetic include cosmic, gamma and X-rays. X-rays were the first to be used to induce mutations and gamma radiation from radioactive cobalt (^{60}Co) is widely used. It has high penetrating potential and is hazardous. However, it can be used for irradiating whole plants and delicate materials, such as pollen grains.

Use of gamma radiation

The use of radiation technique with *in vitro* propagation over plants to out numbered vegetatively is an effective technique in terms of obtaining variation, quick proliferation of mutants and obtaining disease-free mutants. This combination has been successfully applied in date palm, apple and pineapple (Anonymus, 2001). Sutarto *et al.*, (2009) with their 20 Gy and 40 Gy gamma application, determined two mandarin and one pummelo species to have potential in terms of being seedless plants and offered field practices over these species. Yoshioka *et al.*, 1999 reported that gamma radiation induced mutants which are resistant to black spot disease, caused by *Alternaria alternata* in Japanese pear, is the most important and serious disease in Japanese pear. Nine resistant mutants were selected from chronically irradiated 'Nijisseiki'. One of these mutants was registered as 'Gold Nijisseiki'. A resistant mutant derived from acutely irradiated dormant scions of 'Shinsui' was registered as 'Kotobuki Shinsui'. Four resistant mutants were selected from chronically irradiated 'Osanijisseiki'. One of them was registered as 'Osa Gold'. Moreover, mutations with a higher level of resistance than that of 'Gold Nijisseiki' were induced from 'Gold Nijisseiki' by acute and chronic gamma-ray irradiation. Khalil *et al.*, (2011) developed sparse seeded mutant of kinnow (*Citrus reticulata* Blanco) using gamma

radiation. Dormant bud scions of Kinnow were subjected to gamma irradiation dose of 20 Gy which was best. The irradiated bud scions were grafted onto Rough lemon rootstock, using the side-graft technique. Russo *et al.,* (1981) carried out induced mutations with gamma rays in seeded 'Monreal' Clementine mandarin (*Citrus reticulate* Blanco) and produced mutants with fewer seeds per fruit. An early flowering putative mutant designated 'GN-60A' was identified among the regenerated plants obtained from gamma ray-treated populations in the glasshouses of the FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, Austria. The GN-60A clone has been micropropagated and its vegetative progeny sent to Honduras, Australia, South Africa and Malaysia for field testing under commercial plantation conditions.

In Malaysia, early flowering plants obtained in the field were tissue cultured again to produce about 2000 plants for commercial evaluation in the United Plantations Bhd. in September 1993. From this population a selection, flowering about 10 weeks earlier than the original parental clone (i.e. Grande Naine), was launched under the name Novaria in 1995. Ram and Majumder (1987) developed a dwarf mutant of papaya known as Pusa Nanha through mutation breeding by treating the seeds of papaya strain Pusa 1-15 with 15 Kr gamma rays (Table 3).

Doses of mutagens

One of the first steps in mutagenic treatment is the estimation of the most appropriate dose to apply. Radiation dose is expressed in rads (radiation-absorbed dose) which is equivalent to absorption of 100 ergs/g. The unit kilo rad (kR which is 1,000 rads) which was in use earlier is replaced by gray (Gy) which is currently used. The two can be interconverted as 1 kR is equivalent to 10 Gy. Neville *et al.,* (1998) proposed the precise method for the determination of absorbed dose of radiation. A concept of LD_{50} (lethal dose 50 %) is used to refer the optimum dose to be used in the experiment. By definition LD_{50} is the dose which causes 50 % lethality in the organism used for irradiation in defined time. It varies with the plant species, the type and status of the material and the stage at which lethality is measured. Generally, irradiated populations are generated by using an LD_{50} dose treatment and with a dose lower than LD_{50}. To obtain a mutant, the dose of the mutagen should be sufficiently high to increase the probability of inducing a mutation.

However, dose should not be so high as to cause damage to the cells or tissues resulting in lethality. Since, induction of mutation is a chance event, and recovery of a mutation is dependent upon chance of the survival of that individual plant, this strategy improves the probability of obtaining a desirable mutant. Radiosensitivity varies with the species and the cultivar, with the physiological condition of plant and organs, and with the manipulation of the irradiated material before and after mutagenic treatment (Briggs and Konzak, 1977; D'Amato, 1992) (Table 2). In plants which are sensitive to radiation, doses lower than LD_{50} are also used to reduce the mutation load (Shu *et al.,* 2012).

Chemical mutagens

Chemical mutagens were found to be highly effective in inducing true gene mutations and the specificity of action could be investigated through analysis of their reaction with different DNA base. There are different chemical mutagens used to induce mutation in fruit crops namely alkylating agents most useful for mutation Ethyl methanesulphonate (EMS), Diethyl sulphate (dES), Ethyleneimine (EI), Ethyl nitroso urethane (ENU), Ethyl nitroso urea (ENH) and azides

(Heslot, 1977). As compared with physical mutagens, chemicals may give rise to relatively more gene mutations rather than to chromosomal changes. The assessment of LD_{50} for chemicals is determined by varying the concentration and duration of treatment, the solvent used (e.g. Dimethyl sulfoxide (DMSO), or the pH of the solution (Novak, 1991). EMS was used for inducing mutations in banana by treating shoot tips and then regenerating adventitious buds. Among chemicals, ethyl methanesulphonate (EMS) belonging to the group of the alkylating agents has been reported as a very effective and efficient mutagen for creating of somaclonal variation in crop plants such as banana and grapes (Omar *et al.,* 1989; Van Harten, 1998; Predieri, 2001; Luan *et al.,* 2006 and Berenschot *et al.,* 2008). Mutation induction in banana has been carried out by subjecting of shoot tips to mutagen followed by regenerating of treated shoot tips (Predieri, 2001). Bhagwat and Duncan (1997) also used chemical mutagens in banana (*Musa* spp. AAA group) to produce variants displaying resistance against fusarium wilt (*Fusarium oxysporum* f. sp. *cubense*). They determined 200 mM of EMS for 30 min as an optimal dose and duration treatment. *In vitro* mutation induction for salt tolerance using EMS was also reported in sweet potato (Luan *et al.,* 2006).

Table.1 Identified spontaneous mutants in fruit crops

Crop	**Original variety**	**Mutant cultivar**	**Nature of mutation and traits**	**Reference**
Mango	Rosado de Lea Davis haven	Rosica Haden	Bud sports Precocious, regular bearer, and larger fruit size Large fruit size	Medina (1977) Young and Ledin (1954)
Banana	Highgate Motta Poovan	Gros michel Poovan	Sports, semi dwarf Sports	Daniells (1990)
Grapefruit	Foster	Hudson	Bud sports, deep red flesh	Soost and Cameron (1975)
Pear	Clapp's Favourite	Starkrimson	Bud sports, spotting of coloured	Bishop (1966)
Mandarin	Owari Pongan	Clausellina Pongan 86-1	Bud sport	-
Navel Orange	Bahia Washington	Baianinha Navelina, Navelate,Marrs, Leng, Autumn Gold, Powell Summer, Winter Red	Limb sports Limb sports	-

(Ray, 2002)

Table.2 Some examples of radiosensitivity of tissue in fruit crops

Species	Mutagen	Plant material	LD50 (Gy)	Reference
Apple	gamma rays	leaves	>10–<20	Predieri and Fasolo (1989)
Japanese plum	gamma rays	Shoots	30	Predieri and Gatti (2000)
Strawberry	gamma-rays	Shoot clumps	>50–<100	Jain (1997)
Kiwi	gamma-rays	leaves	40–60	Sammarcelli-Ollitraut and Legrave (1991)
Banana	gamma-rays	Shoot tip	30–35 (Triploid)	

Table.3 Achievements through mutation breeding

Species	Cultivar released	Year	Mutagen	Trait improved
Apple	Golden Haidegg	1986	Gamma rays	Large fruit size
	Lysgolden	1970	Gamma rays	Rust resistance
	Courtavel	1972	Gamma rays	Shortness
	Senbatsu-Fuji-2-Kei	1985	gamma rays	Fruit colour
Peach	Mangnif 135	1986	Gamma rays	Fruit size
	Plovdiv 6	1981	Gamma rays	yield
Banana	Novaria	1993	Gamma rays	Earliness
	Klue Hom Thong KU1	1985	Gamma rays, *in vitro*	Bunch size
Orange/ mandarin	Valencia 2 INTA	1987	X-rays	Fruit set, quality
	Eureka 22 INTA	1987	X-rays	Fruit set, quality
Papaya	Pusa Nanha	1986	Gamma rays	Dwarfness
Pear	Kotobuki shinsui	1996	Gamma rays	Disease resistance
Apricot	Early Blenheim	1979	ThN	Earliness
Grape	Fikreti	1986	Gamma rays	Earliness
Almond	Supernova	1987	Gamma rays	Fruit size
Plum	Spurdente-Ferco	1988	Gamma rays	Earliness
Sour cherry	Plodorodnaya Michurina	1977	X-rays	Fruit set
	Plodorodnaya Orlovskoi	1979	Gamma rays	Dwarfness
	Karlik Samorodka	1979	Gamma rays	Dwarfness
sweet cherry	Burlat C1	1983	Gamma rays	Compact growth
	Ferrovia spur	1992	X-rays	Dwarfness
	Compact Stella 35B11	1974	X-rays	Compact growth

(Predieri, 2001).

Table.4 DNA markers for genetic diversity assessment in fruit crops

Fruit	Marker Type	References
Apple	AFLP and RAPDs	Coart *et al.*, (2003); Botez *et al.*, (2009); Sestras *et al.*, (2009)
Avocado	Mini satellite DNA	Ashworth *et al.*, (2003)
Banana	RAPDs	Brown *et al.*, (2009)
Citrus	RFLP	Durham *et al.*, (1992)
Pistachio	Mini satellite marker	Riaz Ahmad *et al.*, (2003)
Pear	SSRs and AFLP	Sisko *et al.*, (2009)
Cashew	RAPD and ISSR	Thimmappaiah *et al.*, (2009)

Colchicine treatment

Colchicine has been used on small fruits with the aim of improving yield parameters (e.g. fruit size) or for using polyploids in cross-breeding. Colchicine has been widely used to increase ploidy level (Van Harten, 1998). This is of vital importance for banana cross-breeding (Van Duren *et al.,* 1996). Colchicine was used for inducing ploidy doubling in octoploid tissue cultured strawberry (*Fragaria* × *ananassa* Duch.) (Predieri *et al.,* 1989). Shoots were treated in a liquid solution with concentrations ranging from 0.65 to 12.5 mM for periods varying from five to nine days. Colchicine was used to obtain tetraploids of banana by immersing shoot tips of the diploid clone 'SH- 3362' in a liquid MS medium (Murashige and Skoog, 1962) containing 0–1% (2.5 mM) colchicine. Stable autotetraploids were established in the field, although some chimerism and reversion to diploid were recorded (Hamill *et al.,* 1992). Wu hu *et al.,* (2012) reported that colchicine-induced red-fleshed *Actinidia chinensis* autotetraploids produced from three female selections ('Hort 22 D', Selection 1 and Selection 2) and one male (Selection 3). Sun *et al.,* 2009 reported that a European pear (*Pyrus communis* L.) cultivar 'Fertility' was evolved by in vitro colchicine treatment of leaf explants.

Somaclonal variation

Somaclonal variation has led to the selection of several variants with increased resistance to pests, diseases, and herbicides (Brar and Jain, 1998), and can be considered as a tool for inducing variation in fruit improvement (Bouharmont, 1994; Karp, 1995; Hammerschlag *et al.,* 1995). In strawberry, somaclones resistant to *Fusarium oxysporum* f. sp. *fragariae* (Toyoda *et al.,* 1991), to *Alternaria alternate* (Takahashi, 1993), and to *Phytophthora cactorum* (Battistini and Rosati, 1991) have also been reported. Similarly in apple rootstocks M26 and MM106 (*Malus pumila* Mill.). Peach somaclones resistant to the root-knot nematode (*Meloidogyne incognita* Kofoid and White) have been obtained as well (Hashmi *et al.,* 1995). Khalil *et al.,* (2011) produce Bingtang sweet orange somaclones tolerant to citrus canker disease by *in vitro* mutagenesis with 0.5 % of EMS. Somaclones resistant to canker disease was later known as DG-2. Kuksova *et al.,* (1997) reported that grapevine somaclones possessing field resistance to *Botrytis cinerea* and *Plasmopara viticola* have also been found. With respect to the issue of resistance to abiotic stress, genotypes with increased tolerance to salinity were obtained from somaclonal variants in *Citrus* species (Ben-Hayym and Kochba, 1983; Ben- Hayym and Goffer, 1989).

Application of molecular for the detectation of mutation in fruit crops

Before 21st century, mutants were mostly selected by observing phenotypes of individual plants in mutated populations treated with chemical or physical mutagens. Concurrently, various molecular and genomics tools have been developed for the detection of genetic variants including single nucleotide polymorphism (SNP). The integration of genomics information and molecular tools has resulted in the development of various molecular approaches that can be use in the screening for mutants in large populations derived from radiation and chemical mutagenesis.

Techniques

DNA marker techniques can also be used widely in plant mutation breeding and genetic research for increasing both the efficiency and efficacy of mutation techniques. They can be used for tracing the pedigree of induced

mutants and tagging important mutations. Consequently, closely linked markers of mutant traits can be used for MAS, pyramiding and cloning of mutant genes. However, the way they are used is somewhat different from conventional plant breeding programmes. Some biochemical markers such as proteins and enzymes can be used as efficient markers in crop improvement, AFLP is a technique that combines aspects of both RFLP and DNA amplification using the polymerase chain reaction (PCR). AFLP typically allows the analysis of dozens of DNA markers simultaneously. Combinations of primers with alternate supplementary bases allow many different sub-sets of DNA marker combinations, or genomic representations, to be analyzed, a feature that makes this technique very versatile. Like RAPD markers, AFLP markers are either present or absent. AFLP is normally used as a genetic fingerprinting method or when large numbers of markers are required from which candidate markers linked to a target can be selected.

For sheer density of DNA markers, single nucleotide polymorphisms (SNPs) offer one of the richest sources. SNPs are increasingly used as DNA markers because techniques are becoming more widespread, cost effective and automated and the genome sequence information of major crops are becoming increasingly available for use. Since many naturally occurring mutations are single nucleotide polymorphisms (SNPs), various technologies have been developed for SNP detection, most of which are also useful for the detection of small insertion/deletions (indels). They can be used for tracing the pedigree of induced mutants and tagging important mutations. Consequently, closely linked markers of mutant traits can be used for MAS, pyramiding and cloning of mutant genes. However, the way they are used is somewhat different from conventional plant breeding programmes. Some biochemical markers such as proteins and enzymes can be used as efficient markers in crop improvement, AFLP typically allows the analysis of dozens of DNA markers simultaneously (Table 4). Combinations of primers with alternate supplementary bases allow many different sub-sets of DNA marker combinations, or genomic representations, to be analyzed, a feature that makes this technique very versatile. Like RAPD markers, AFLP markers are either present or absent. AFLP band intensities can be quantified and scored as co-dominant marker, especially between samples of closely related individuals such as backcross populations to discriminate heterozygous loci.

Mutation is important breeding tool for creating variation in fruit crops. It provides an oppurtunity 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 help in development of commercial varieties in achieving the target of nutritional security.

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