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Mutation for Disease Resistance in Fruit Crops- A Review

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ABSTRACT: Mutation breeding techniques are gaining attention in crop improvement which can create desirable variations in the genetic base of plants. Well adapted plant varieties can be improved by modifying one or two major traits, which limit their productivity or quality. Different viral, bacterial and fungal diseases are causing a huge loss in fruit crops, by reducing production and quality. Mutation breeding can be used as an eco-friendly and inexpensive method by creating disease-resistant plants. There are different barriers in fruit breeding and induced mutations help to break these obstacles. Several experiments have been conducted in this area worldwide and several disease-resistant mutant varieties are released for cultivation such as McIntosh 8F-2-32 variety of apple, Smile Heart variety of strawberry and US Furr ST variety of mandarin. The application of induced mutations along with biotechnology, genomics and molecular marker techniques, can speed up fruit breeding. In this paper, induced mutation studies for developing disease resistance in major fruit crops so far are being discussed. In addition, disease-resistant somaclonal variants in different fruit crops are also studied.

Keywords: Induced Mutation, Disease Resistance, Fruit crops.

INTRODUCTION

Mutation is defined as a sudden heritable change in the genetic material, which is not caused by recombination or segregation (De Vries, 1901). It is creating a renaissance in crop improvement by introducing novel changes in the genetic make-up of crops. The findings of X-ray induced mutations in fruit fly (Muller, 1927) and barley (Stadler, 1928) enlightened the ways of using irradiation for crop improvement. Mutation breeding aims at the creation and selection of new traits which increases the range of alleles available in crops, whereas, the conventional breeding program only utilizes the existing genomic resources. Since induced mutations change only one or a few specific traits of an elite cultivar without upsetting the other characters, plant varieties can be improved by altering the undesirable traits which limit their production. It creates desirable variations in plants such as dwarf stature, earliness, seedless fruits and resistance to various diseases and pests within a short period. The first induced mutant variety was released in Indonesia in mid -1930s, which was a light green mutant of tobacco. Thereafter, quite a few numbers of mutant crop cultivars with improved characteristics have been developed. Mutation is getting increased attention as the International Atomic Energy Agency along with the Food and Agriculture Organization, has developed a Mutant Variety Database which describes officially released plant mutant varieties throughout the world. It presently includes more than 3320 mutant varieties in 228 different plant species from more than 73 countries (FAO/IAEA MVDB, 2016).

Mutation breeding is also gaining attention in horticulture as conventional breeding in horticultural crops is facing different barriers such as long vegetative phase, heterozygosity, polyploidy, incompatibility, apomixes and sterility. Induced mutations can be effectively used to take care of these barriers in fruit breeding (Suprasanna and Nakagawa 2012). Mutation in fruit crops has existed for many years, as natural bud mutations form a large number of commercially important cultivars in fruit crops. Since the frequency of natural mutations is very low, induced mutagenesis can be used as a solution to increase the frequency manyfolds. One of the earliest attempts of induced mutations in fruit crops was X-ray irradiation in apple scions (Stadler, 1930). After that, many successful mutants were released demonstrating the potential of induced mutations in fruit breeding such as deep red coloured apple (Bishop, 1959), dwarf papaya named Pusa Nanha (Ram, 1983), seedless citrus, rust-resistant apple *etc*.

Diseases in plants are a major threat to the yield, quality and economical value of the crops. Fusarium wilt in banana and papaya ringspot are some of the many diseases which are very difficult to control. The development of resistant lines through induced mutations, which creates disease resistance in plants rapidly, is an economical and eco-friendly measure to deal with this problem. Mutagenesis complemented with molecular techniques, such as molecular markers

and high throughput mutation screening, are more powerful and effective in crop improvement (Shu, 2009). Several studies have been conducted in this field to develop disease-resistant mutants such as scab tolerant mandarin mutants (Gasic *et al*., 2014), wilt resistant mutants in banana (Hegde *et al*., 2019) and Alternaria blotch resistant mutants in apple (Saito *et al*., 2000). Somaclonal variations in tissue culture also contribute resistance against several diseases such as Verticillium wilt in strawberry (Zebrowska, 2010), blight in apple (Donovan *et al*., 1994) and Fusarium wilt in banana (Lee *et al*., 2011).

In this paper, we discuss the achievements of disease resistance using mutagenesis in major fruit crops such as banana, apple, citrus, grape, etc. The different mutagens used, methodologies followed and their results are examined so that the impact of mutagenesis in resistance breeding of fruit crops can be realized. Somaclonal variations that lead to disease resistance in fruit crops are also being discussed.

MUTAGENS

Mutagens are the agents causing mutation. There are different physical and chemical mutagens used in plant breeding. Physical mutagens include non-ionizing radiations (UV rays) and ionizing radiations (X-rays, gamma-rays, alpha, beta particles, electron beam laser and fast neutrons). X-rays and gamma-rays are the most expedient radiations for application and usage. The advantages of physical mutagens are high and uniform penetration (except for UV rays), good dosimetry and better reproducibility. The common chemical mutagens are Alkylating agents (mustard gas, EMS, MMS), Base analogues (5-chlorouracil) and Acridine dye (acridine orange, proflavine). These are easily available at low cost and result in a high mutation rate but are hazardous to use and disposal is difficult. Chemical mutagens such as ethyl methane sulphonate and ethylene imine induce more mutations at some loci than physical mutagens such as X-rays or gamma-rays (Amano, 1972). Fixing the dosage of mutagen is the preliminary step in mutation, which is done by LD 50 concept. LD 50 (lethal dose 50 %) is defined as the dosage which causes 50 % lethality in the organism used for irradiation at a distinct time. It changes with the plant species, the nature of the material and the stage at which lethality is determined. Donini (1975) reported that the LD50 value in dormant scions of raspberry was 50 Gy gamma-rays from his induced mutation studies using gamma-ray doses ranging between 20 and 60 Gy. Thus the selection of mutagens and fixing their dosage is very important in mutation breeding.

INDUCED MUTATION FOR DISEASE RESISTANCE

Plant diseases reduce the yield and quality of crops and intimidate food security. The appropriate measure to control diseases is the development of resistant lines. As conventional breeding methods take much time, larger space and more labour, induced mutations create disease resistant plants easily and rapidly. Mutations in the susceptibility gene reduce the compatibility between hosts and pathogens and create resistance against pathogens (Li *et al*., 2020). Thus several disease resistant mutants are released in different fruit crops, which are discussed below.

Banana. Banana is one of the important fruits in our country. Conventional breeding techniques in banana are difficult due to its seedlessness, sterility and triploidy. Most of the banana export cultivars grown today are selections from Cavendish somatic mutants (Perrier *et al*., 2011). Nowadays induced mutations are widely utilised in banana breeding. The LD50 value of gamma rays in micropropagated bananas was established at 40 Gy. The shoot proliferation was stopped beyond 50 Gy and a dose of 70 Gy was completely lethal for all the genotypes examined (Rao *et al*., 1998). Smith *et al*. (1985) used 20Gy gamma irradiation in banana and retained the resistance to race 4 fusarium pathogen shown by the mother plant Dwarf Parfitt. Due to the heterozygous nature, a large population has to be maintained for screening. Several methods have been developed for the rapid screening of resistant banana mutants. As a comparative study for resistance to black leaf streak using in vitro plantlets in tubes and detached Leaves, symptoms appeared much earlier in vitro plantlets than in detached leaves. (Twizeyimana *et al*., 2007). Tripathi *et al*. (2008) also reported that in vitro screening test is a convenient, cheap and rapid screening technique to select Xanthomonas wilt-resistant banana cultivars. Pot system and hydroponic systems are the commonly used bioassay for screening wilt resistant plants and in vitro bioassay is a fast resistance diagnosis method that increases the banana breeding efficiency (Wu *et al*., 2010). Nowadays molecular techniques are used for the rapid detection of mutants. Hegde *et al*. (2019) used an inter-simple sequence repeat (ISSR) marker to detect Fusarium wilt resistance in banana. They obtained 109 amplified ISSR fragments of which 8 were polymorphic. Reformed genetic profiles compared to control and susceptible were recognised in 25 Gy and 30 Gy, moderately resistance in 35 Gy and 40 Gy and resistance in 40 and 45 Gy mutants. ISSR marker is an efficient tool for the early detection of mutants.

In banana breeding, the priority is to develop plants resistant to Fusarium wilt, which is the most serious problem in banana. Bhagwat and Duncan (1997) screened wilt tolerant banana plants using chemical-induced mutation. They found no substantial differences between the numbers of variations induced by the three mutagens namely sodium azide, diethyl sulphate, and ethyl methane sulphonate and their durations. They perceived 4.6, 1.9 and 6.1% of plants regenerated after treatment with sodium azide, diethyl sulphate and ethyl methane sulphonate respectively exhibited less than 10% vascular invasion of their corms without any external symptoms. These tolerant plants were further evaluated but failed to get the same level of tolerance in all the locations. Later, several mutation studies have been conducted using chemical mutagens in banana. Chen *et al*. (2012) illustrated EMS-induced mutation of Brazil banana through the micro-cross-section cultural system is effective for crop improvement. They determined the optimum dose of

EMS as 300 mm for 60 minutes. *Fusarium oxysporum* f. sp. cubense race 4 pathogen was recognised in the preliminary test field with a SCAR marker. Five Fusarium wilt-resistant lines were screened which showed markedly reduced disease incidences compared with the control. Kishor *et al*. (2017) screened three wilt resistant lines with the treatment NaN_3 at 0.01%. Explants of banana cv. Nanjanagudu Rasabale from shoot tip culture were treated with various concentrations of EMS and NaN_3 and the putative mutants were artificially inoculated with *Fusarium oxysporum* f. sp. Cubense and screened. They stated that EMS treated mutants were found more susceptible compared to NaN_3 treatment. The use of gamma irradiation for the development of wilt resistance was also found productive. Hegde *et al*. (2019) showed that gamma-ray derived mutants at 40 and 45 Gy were resistant to fusarium wilt in Nanjanagudu Rasabale and Ney Poovan banana. The mutants from 25, 30 and 35 Gy were highly sensitive. SSR primers displayed resistance specific bands and were used for analysing variations. Recognised resistant, moderately resistant and susceptible mutants along with control banana plants showed the presence of a major band 150 bp and 500 bp in Nanjanagudu Rasabale and 500 bp and 250 bp in Ney Poovan. This supported the presence of random mutations in the genome. From the above works, we can say induced mutagenesis is an appropriate solution for the wilt problem in banana.

Viruses such as Banana bunchy top virus, Banana bract mosaic virus and Banana streak virus are deleterious in banana and are very difficult to control. Quite a few efforts were taken in developing mutants resistant to these viral diseases. Banana bunchy top virus is one of the devastating diseases in banana which is spread by banana aphids. Imelda *et al*. (2000) selected BBTV tolerant mutants in vitro. The shoots of banana cultivars Ambon hijau, Barangan and Raja sere were treated with EMS (0.7% for 2-4 hours). Eman *et al*. (2012) investigated mutation induction in shoot cultures of banana for resistance against BBTV and BMV. Chemicals such as 2, 4-D (2, 4 and 6mgL-1), 6-Benzyl amino purine (6, 7 and 8 mgL-1) and Sodium azide (1, 2 and 3 mgL-1) were used for induction of mutation. Banana plants were screened using the syringe method of inoculation. They isolated two banana plants treated with 6mgL-1 2, 4-D, which showed resistance against BMV, but they failed to obtain resistance to BBTV. Damasco *et al*. (2019) gamma-irradiated the banana cv. Lakatan and ten banana bunchy top virus-resistant mutants were selected. High level of polymorphism was observed in the mutants using SSR analysis. Mutant lines were differentiated from the control plants by the absence of one or few alleles in mutant lines with four primers and/or the addition of one or few alleles in mutant lines with two primers.

Sigatoka leaf spot is the most common disease found in banana worldwide. Reyes-Borja *et al*. (2007) suggested that a carbon-ion beam at 8 Gy could be useful for inducing mutation in vitro plantlets of banana cultivars 'Cavendish Enano' and 'Williams'. The survived plantlets were planted in the field for evaluation in which, six plants from the 'Williams' population and two plants from 'Cavendish Enano' population were found resistant to black Sigatoka. Glance on these experiments is revealing the potential of mutagenesis in banana resistance breeding. More mutation works utilizing the molecular markers have to be conducted in banana for creating plant resistance.

Apple. The earlier mutation studies in apple were focused on the development of russet free and deep red coloured fruits. Bishop (1959) noticed resistance against powdery mildew in thermal neutron induced colour mutant of Cortland, Nova Red. One of the pioneer studies in mutation for disease resistance in apple was done by Mcintosh and Lapins (1966). They irradiated the dormant buds of apple cv. McIntosh and were grafted onto 10 year old trees, re-propagated by budding on seedling rootstocks. Thus, the third vegetative generation from treated shoots was under observation and they exhibited a range of reduced susceptibility to powdery mildew. They also reported that there was no correlation between the compactness of the tree and mildew susceptibility. The mutant variety McIntosh 8F-2-32 produced by irradiation of shoots with gamma rays was officially approved in 1970. Which was found resistant to Podospheraleucotricha, Venturiainaequalis and was showing improved skin colour (FAO/IAEA Mutant Variety Database).

Alternaria leaf blotch disease caused by *Alternaria mali* has been a serious issue in apple for several decades. Alternaria blotch resistant apple mutant was reported by Masuda and Yoshioka in 1996. A single dominant gene is responsible for the susceptibility to the disease which was eliminated using irradiation. The susceptible variety 'Indo' is heterozygous for the locus. The mutants were selected out of 3002 MI V 4 shoots propagated from gamma-irradiated shoots with a dose of 80 Gy (5 Gy/h) in vitro. The putative mutants showed intermediate resistance to the disease. Later Tabira *et al*. (1998) also screened mutant strain (ID-120-S40) resistant to Alternaria blotch in apple and noticed the differential expression of a protein in the susceptible cultivars by 2-D gel electrophoresis. The apical meristem cultures of susceptible cv. Indo was subjected to gamma-rays and mutants were screened using AM-toxin I. The results of two-dimensional gel electrophoresis indicated that the protein spot of 60 kDa with pI 5.5 was detected in nine susceptible strains and was not detected in twelve resistant strains inspected. Fukasawa-Akada *et al*. (1999) crossed resistant and susceptible varieties of apple and conducted bulked-segregation analysis of the F1 population to identify the RAPD markers linked to susceptibility to Alternaria blotch. These markers were found in Hokuto cultivar but were absent in resistant mutants of irradiated cv. Hokuto (Fukasawa- Akada *et al*. (1999) unpublished results). Saito *et al*. (2000) also studied mutants resistant to alternaria blotch in different cultivars of apple. They irradiated in vitro cultured apple shoots with X-rays and gamma rays and screened them with chemically-synthesized AM-toxin I of *Alternaria alternata* (Fr.). The development of symptoms was studied using spore suspension of A. alternate. They isolated 4 resistant mutants by X-ray irradiation (10

KR) in cv. Hokuto. They obtained 13 resistant mutants by X-ray irradiation and 20 resistant mutants by acute gamma-ray irradiation in cv. Fuji, and 12 resistant mutants by acute X-ray irradiation in cv. Oorin.

Citrus. Traditional breeding of citrus becomes a cumbersome process because of its biological barriers such as sexual incompatibility, polyembryony, heterozygosity and sterility (Camerone and Frost, 1968). Tulmann *et al*. (1990) worked in sweet orange cv. Pera. They irradiated the buds with 40 Gy gamma rays and grafted on onto rootstocks. The mutated shoots were pruned, divided into quadrants and observed under field conditions. In some cases, the whole plant exhibited tolerance to citrus canker disease and in others only one quadrant exhibited tolerance. Later, Latado *et al*. (2006) identified two mutant lines tolerant to citrus canker disease in sweet orange cv. pera through gamma ray irradiation. The tolerance was found consistent over five years in field conditions but they had lower fruit yield. One of the first reports of successful disease resistant mutants in citrus was done by Belasque *et al*. (2009). They studied six mutant clones of sweet orange cv. Pera for their resistance against citrus canker and was compared with three different varieties. They found three gamma ray irradiated mutant clones (9-1, 9-2 and 9-3) with higher resistance. They exhibited a lower incidence of disease, the longest period of incubation of the disease, a smaller diameter of the lesion and a lower area under the disease progress curve. The resistant mutants were found comparable with the most resistant cultivar, Ponkan mandarin under conditions of artificial inoculation. Thus gamma irradiation is found promising in producing canker resistant citrus varieties.

Mutation breeding in lemon started for developing seedless and mal secco tolerant cultivars. Gulsen *et al*. (2005) carried out bud wood irradiation in Kutdiken lemon clone KT-2A using gamma rays and suggested LD50 dosage as 5 krad. They screened stable seedless and mal secco tolerant plants by 5 and 7 krad irradiation. Altered tree morphology and early maturation of fruits were observed with 7 krad irradiation. A new variety of mandarin named US Furr ST tolerant to scab was developed using irradiation and released for cultivation. (Gasic *et al*., 2014).

Grape. Donini (1975) used gamma-rays in grape and fixed the LD50 dosage as 30 Gy. It resulted in mutants with short internodes, early fruit maturity, seedlessness and resistance to berry fall in V. vinifera cv. 'Bonarda', and 'Regina deiVigneti'. Similar studies were conducted by several scientists worldwide, to fix the LD 50 value in grape. Surakshitha *et al*. (2017) studied the gamma-ray induced mutation in hardwood cuttings of grapes. They worked out the LD50 value of gamma rays to be 15–20 Gy for 'Red Globe' and 15–25 Gy for 'Muscat' based on the survival and growth rate of treated cuttings. Different methods were developed to screen the disease resistant mutants in grape. As a comparison of dual culture and resveratrol production, Barlass, (1985) suggested shoot dual culture as an efficient technique to select resistant mutants against the obligate parasite, *plasmopara viticola*.

Papaya. Yeh and Gonsalves (1984), worked on papaya ring spot virus and isolated two mutants, designated PRV HA 5-1 and PRV HA 6-1, which showed diffused mottling without reduction in plant size. PRV HA 5-1 showed protection against a severe PRV strain under greenhouse conditions. They endorse symptomless mutant could be used as a protectant for control of PRV. Several other mutation experiments have been conducted in papaya (Pujar *et al*., 2019; Sahu *et al*., 2019) but significant results in developing disease resistance are not yet reported.

3.6 Other fruit crops

Black spot disease, caused by *Alternaria alternata* Japanese pear pathotype, is the most devastating disease in Japanese pear. A single dominant gene controls susceptibility to this disease. Yoshioka *et al*. (1999) carried out gamma ray induced mutation in Japanese pear and nine black spot disease resistant mutants were selected. Gold Nijisseiki selected from Nijisseiki and Kotobuki Shinsui derived from Shinsui. Osanijisseiki, one of the resistant mutants showed unfavourable characteristics but four resistant mutants were screened from this and one of them was registered as Osa Gold. Kotobuki Shinsui variety developed by irradiation with chronic gamma rays (80 Gy) was exhibiting resistance to black spot and was officially approved in 1996 (FAO/IAEA Mutant Variety Database).

Ibrahim *et al*. (2009) used gamma-irradiation on pineapple cv. Josapine, which is susceptible to bacterial heart rot and developed resistant mutants with improved quality. They screened resistant plants with increased total sugar content and fruit weight at higher doses of 30 and 40Gy. Further studies on molecular analysis using AFLP technique can be carried out to identify markers for the particular characters.

Bayoud disease caused by *Fusarium oxysporum* f. sp. albedinis an epiphytic disease seen in date palm. Gamma irradiation of somatic embryogenic cell cultures of date palm reported tolerance to this destructive disease at the field level. Regenerated plants were treated with Bayoud toxin in the greenhouse, putative mutants tolerant to Bayoud disease were evaluated at the field level (Jain, 2012).

Sangameshkolavi (2014) screened pomegranate mutants with moderate resistance to bacterial blight, with the gamma ray treatments at 10 Gy, 15 Gy, 20Gy and 25 Gy. As bacterial blight is the most devastating disease in pomegranate, studies for the development of blight resistant plants using EMS and gamma rays are in progress.

Although most of the mutation studies have been done in field crops, researches in horticulture crops are gaining interest. As conventional fruit breeding takes too much time, mutation techniques have to be more encouraged which gives results rapidly. Moreover, it is an environment friendly method safe for humans and other organisms. Any foreign gene is not transferred to the crops which avoids the risk. Mutation works should be extended for the development of crop varieties that can grow in marginal lands and can cope with changing climate.

SOMACLONAL VARIATIONS

Somaclonal variations, the term used by Larkin and Scowkraft (1981) for plant variants, are instrumental in crop improvement. It utilizes the variations in tissue culture regenerated plants of somatic cells. The possible factors which affect the variations are and age and source of explants, sub-culture frequency, duration, culture environment, chemical additives, growth stimulants, media composition, level of ploidy, genetic mechanism *etc*. (Silvarolla, 1992). These variations may be genetic or epigenetic. Somaclonal variations are not only found in asexually propagated crops but also found in seed propagated species. Pre-existing differences in the somatic cells of the explants and those generated in culture lead to somaclonal variations in vitro (Nwauzoma and Jaja 2013). Even though these variations are useful in plant breeding, they are undesirable in clonal propagation and germplasm conservation.

Numerous studies have been conducted in different crop species using somaclonal variations after the study of morphological variants in sugarcane plants in vitro was reported (Heinze and Mee 1971). It is a potential tool for obtaining genetic variability relatively rapidly and without sophisticated technologies in horticultural crops, which has a narrow genetic base (Krishna *et al*., 2016). Fruit trees are perennial crops that require a larger area and a longer time for breeding. In contrast, somaclonal variations in vitro consume less space and screening time. These somaclones can create various desirable changes in plant traits such as morphological characters, disease resistance and maturity time. The frequency of variation detected within different sets of multiple regenerants resulting from single embryos in peach was different, which reiterates that the frequency of somaclonal variation is genetically determined (Hammerschlag and Ognjanov 1990). Micropropagation and induced mutagenesis in vitro can increase the recovery of somaclones (Afrasiab and Iqbal 2010). Somaclonal variations coupled with induced mutations can be utilized in fruit breeding for creating disease-resistant plants which are being discussed here.

SOMACLONAL VARIATIONS FOR DISEASE RESISTANCE IN FRUIT CROPS

Somaclonal variations are inducing disease resistance in several plant species. These clones can be screened, tested and released as new varieties for cultivation. Several techniques have been developed for the screening and selection of putative clones. Some of the successful somaclones in fruit crops with disease resistance are listed below.

Banana. Around 1990, a Taiwan farmer named Chung Hwang identified some wilt-resistant plants from the suckers of tissue culture plantlets of Giant Cavendish banana. This clone had better horticultural traits and marked resistance to Fusarium wilt (Hwang and Ko, 2004). It was a successful somaclone with pronounced resistance and higher yield later designated as GCTCV-218. In china, Tai-Chiao No. 5', a somaclonal variant of 'Tai-Chiao No. 3 banana was released as a wilt resistant cultivar in 2007. The horticultural traits and reaction to foc 4 pathogen of Tai-Chiao No. 5 were observed with Pei-Chiao variety as check in three trials. Only 5-25% of the Tai-Chiao No. 5 plants were wilt affected whereas the plants of Pei-Chiao showed 10-50% disease incidence. The 7-year successive evaluation trials exhibited the stable resistance of 'Tai-Chiao No. 5' to Foc race 4 (Lee *et al*., 2011). These varieties gained more consideration since in most of the cases disease-resistant clones fail in better yield and quality traits. Apart from wilt, black sigatoka disease is also causing a huge loss in banana cultivation in several parts of the world. In Nigeria, a somaclone called AO 2B2-2 derived from Agbagba variety of banana showed tolerance to black Sigatoka disease. AO 2B2-2' had a higher bunch weight, more fruits per bunch with higher average length and girth than Agbagba (Nwauzoma *et al*., 2002).

Strawberry. In strawberry, several disease-resistant somaclonal mutants have been released for cultivation such as variety Smile Heart resistant to phytophtora rot (*Phytophthora nicotinae*), officially approved in 1991 and variety Akita Berry resistant to black leaf spot disease (*Alternaria alternata*) was officially approved in 1992 (FAO/IAEA Mutant Variety Database). Kaundal *et al*. (2003) reported that in strawberry cv. Chandler, more variants were observed in somaclones (2.46%) than in irradiated plants (0.76%). The frequency of occurrence of the variants was found to be genotype-dependent as it was higher in irradiated plants of cv. Fern (2.07%) than that of cv. Chandler. Zebrowska (2010) worked in strawberry somaclones in sterile culture and reported that somaclone of Merton Dawn was more genetically resistant to infection by V. dahliae than somaclone of Selva. Sowik *et al*. (2015) studied the genetic stability of strawberry cultivars Elsanta, Senga Sengana and the somaclone K40. The plants were propagated in vitro for 45 generations and clonal propagated from runners for 4 generations. K40 clones were more resistant to Verticlliumdahliae in the greenhouse than the cv. SengaSengana, which is verticillium wilt resistant in the field. This expounds that the somaclonal variations are stable and transferable through in-vitro micropropagation and clonal plant propagation with runners.

Apple. Somaclonal variations exhibited blight resistance in apple cv. Greensleeves. In the glasshouse-grown plants, 60% of the most promising somaclones showed minimum symptoms after inoculation compared with 4% of 'Greensleeves' parental plants (Donovan *et al*., 1994). Later field behaviour of apple somaclones and different techniques of assessment of fire blight resistance was studied with a more virulent strain of E. amylovora and with more replications. The greenhouse inoculation method was found more reliable in this study (Chevreau, 1998).

Pear. Somaclonal variations in Pear rootstock 'old home × farmingdale' (OHF 333) were studied by Nacheva in 2014. The rootstock was artificially inoculated with a local strain of *Erwinia amylovora* in which 50% of plants exhibited a low degree of visible symptoms. Only 10% of plants showed a high degree of visible symptoms. Twelve fire blight tolerant plants

were screened and planted in the nursery for field evaluation.

Grape. Kuksova *et al*. (1997) analyzed the somaclonal and in vitro mutagen-induced variabilities in grape cv. Podarok Magaracha. They regenerated plants from leaf explants through somatic embryogenesis and were gamma irradiated. They screened 8 somaclones resistant to B. cinerea, 2 somaclones resistant to P. viticola and one diploid somaclone highly resistant to both diseases. By the perusal of this study, we can conclude, the use of induced somatic embryogenesis along with gamma-ray mutation produces desirable somaclonal variants in grape.

Citrus. Malsecco disease in citrus is a tracheomycotic disease caused by Phomatracheiphila. Tolerant somaclone named Feminello-S was selected using partially purified toxin of fungus in vitro (Gentile *et al*., 1992). The in vivo response to artificial inoculation of two somaclones (FS01 and FS11) were studied and compared with the susceptible Femminello and tolerant Monachello lemons. These genotypes were grafted onto sour orange and were stem inoculated. The xylem colonization was observed using PDA plating and PCR analysis. Somaclone named FS01 showed tolerance similar to Monachello (Gentile *et al*., 2000). Hongjuan *et al*. (2019) developed tolerant somaclones of sweet orange cv. Bingtang against citrus canker by in vitro mutagenesis. Cell suspension of embryogenic callus was treated with 1.5 % EMS for 1 hour (the lethal concentration). An Xcc-crude extract isolated from pathogen was used for screening and a canker disease resistant somaclone named DG-2 was successfully isolated.

Numerous reviews have summarised the use of somaclonal variations in disease resistance of fruit crops. As we discussed, somatic embryogenesis coupled with induced mutations found effective in developing disease resistance in different fruit crops. Pear somaclones resistant to *Erwinia amylovora* (Viseur, 1990), mango resistant to *Colletotrichum gleosporiensis* (Litz *et al*., 1991), strawberry resistant to *Fusarium oxysporum* (Toyoda *et al*., 1991), *Alternaria alternata* (Takahashi *et al*., 1993) and *Phytophthora cactorum* (Battistini and Rosati 1991) have been reported. Somaclonal variations are a potential tool in fruit breeding that gives rapid results without any refined technologies. There are some drawbacks since somaclonal variations are unpredictable in nature, its inheritance should be tested. It may cause both positive and negative variations in plants that have to be screened. Sales *et al*., (2016) elucidated that, prolonged subculture and the addition of higher concentrations of 2, 4-D induced different variations in banana cv. lakatan. Positive variations such as higher bunch weight, and longer shelf life and negative variations such as dwarfism, delayed flowering and a lesser number of hands. In horticultural crops, several techniques were developed for the selection of resistant somaclones still there are no in vitro selection methods established for complex traits such as yield, total soluble solids, sweetness, texture or shelf life (Evans, 1989). Despite all these hindrances, somaclonal variations are widely used in crop improvement studies utilizing induced mutations and biotechnological tools in vitro.

CONCLUSION

The development of disease resistance is one of the major goals in fruit breeding since diseases are causing huge losses in different fruit crops. As fruit breeding is being constrained by various limitations, new methods need to be established to develop new varieties. The perusal of different mutagenesis experiments elucidates the importance of induced mutations in fruit crops. In vitro culture combined with induced mutation and molecular techniques speed up the breeding programme. Several disease resistant mutant varieties have been released in different fruit crops which is a breakthrough in fruit cultivation. The use of sequencing techniques helps to identify target-specific genes and easily detect mutations. Nowadays induced mutations are also used to determine the gene/allele function in plants. Mutation studies are to be focused on developing climate-resilient crops as climate change is seriously affecting crop production. Induced mutations coupled with biotechnology and molecular techniques can create better adaptability and higher productivity in crops. Thus mutation breeding coupled with advanced biotechnological tools can be recommended to transform fruit cultivation.

FUTURE SCOPE

Induced mutagenesis is a key technique for generating novel alleles and desired characteristics in crop genomes. The random nature of mutagenesis is one of the major hurdles in mutant breeding. With the help of biotechnological inventions and omics technology, mutation breeding has levelled up to targeted editing. It can be improved further in future and shorten the duration of the study so that induced mutagenesis can create a revolution in enhancing crop production.

Conflict of Interest. None.

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