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ADVANCES IN BIOTECHNOLOGICAL INTERVENTION IN FRUIT CROPS: A REVIEW

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ABSTRACT

The improvement and cultivation of fruit crops have been transformed by advance biotechnology interventions. Biotechnological advancements have revolutionized fruit crop cultivation by offering innovative solutions to longstanding agricultural challenges. This review highlights the significant progress in biotechnological interventions that have been enhanced fruit crop production, quality and sustainability too. Molecular markers have greatly improved breeding programmes accuracy and made it possible to choose desired traits genetically. It used to identify the male and female sex in several fruit crops. CRISPR-Cas9 technology has emerged as a strong genome editing tool, allowing for precise alterations that can increase disease resistance, fruit quality and productivity. Transgenic technologies are being used to add novel features that are difficult to acquire through conventional breeding. While *in vitro* culture techniques are helping to rapidly propagate high-quality, disease-free planting material. These advancements pave the door for the production of fruit crops which are more robust, productive and more adapted to meeting the world's expanding agricultural need. In this review, we highlighted the application of advanced biotechnological approaches utilized in breeding of fruit crops.

Key words : *In vitro*, CRISPR-Cas9, Molecular marker, Genome editing, Transgenic.

Introduction

Fruits are a vital source of fibre, vitamins, and minerals globally (Giovannoni *et al*., 2017). Worldwide fruit production has been enormous, reaching 896.45 million tonnes, with five fruit plants (bananas and plantains, watermelons, grapes, oranges and apples) occupying the majority of the entire production (FAO, 2021). China, India and Brazil have emerged as the leading producers (FAOSTAT database, 2023). The demand for fruits rises in proportion to the population. However, climate resilient is posing significant risks to fruit crop production (Karkute *et al*., 2017). To improve the possibilities of a consistent fruit supply, the pioneers controlling wild plant species into cultivated crops. After the "rediscovery" of Mendel's principles in 1900, breeders began selecting and crossing better plants (Hickey *et al*., 2017). However, while traditional plant breeding techniques have made major improvements in the production of superior cultivars, they have been unable to keep up with the growing demand for fruits. Conventional breeding has numerous major limitations, as it is mostly dependent on existing spontaneous allelic variants. This approach is often ineffective for generating desired traits due to the random mixing of ten to thousands of genes (Karkute *et al*., 2017). Although, traditional breeding has enhanced agricultural yield, it frequently results in a loss of fitness and genetic diversity (Meyer *et al*., 2013). As a result, there is an immediate need to include biotechnology to accelerate agricultural development projects.

Biotechnological techniques have transformed crop development programs by introducing new plant strains, supplying planting material, developing more effective and selective insecticides and improving fertilizers. Fruit crops are traditionally propagated by cutting, budding, grafting,

layering and other methods, but these are a lengthy and complex, often encountering problems such as high heterozygosity, long juvenile phase and auto incompatibility (Petri and Burgos, 2005; Rai and Shekhawat, 2014). It is difficult to provide adequate food supplies for the world's fast rising population (Tester and Langridge, 2010). As a result, present technical innovation is essential to satisfy current customer needs and future thrust (Bigliardi and Galati, 2013). New biotechnological tools (NBTs) such as genetic engineering methods, might encourage the promptly insertion of essential genes into the genome of commercial fruit cultivars, resulting in improved effectiveness and dependability of genetic improvement of clonal propagated plants (Lusser *et al*., 2012), while maintaining high stability of the clone's major traits.

Genetic engineering techniques have wide applications in fruit crops because they allow development of essential agronomic characteristics such as biotic and abiotic stress tolerance as well as fruit quality. These strategies have been used to modify various fruit crops during the last two decades. Plant genetic engineering has been practiced for more than 30 years. The principal approach for introducing heterologous DNA into plants have been direct transformation methods (Biolistic) and indirect methods (*Agrobacterium tumefaciens*-mediated transformation) which were established decades ago (Chilton *et al*., 1977; Gelvin, 2003; Altpeter *et al*., 2005). All commercially developed genetically engineered crops including fruit species, have been produced employing one of these techniques (Parisi *et al*., 2016). Furthermore, because of the high degree of heterozygosity that characterises the majority of these species. It is more agronomically advantageous to regenerate a new fruit tree plant *in vitro* from mature tissues (Cervera *et al*., 1998; Pérez-Jiménez *et al*., 2012).

With respect to conventional breeding, recombinant DNA technology permits transfer of desired genes from any organism, plant or microorganism into fruit crops, expanding possibilities for fruit yield enhancement by providing new genotypes and phenotypes for breeding purposes and ultimately improving fruit quality and shelf life. Thus, genetic engineering has been identified as most rapidly emerging agricultural technology (Parmar *et al*., 2017). Recent biotechnological innovations for producing genetically modified (GM) plants with helpful agronomic and quality features are presently important for many crops (Datta, 2013; Qaim and Kouser, 2013).

The Food and Drug Administration (FDA) certified the transgenic "Flavr Savr tomato" for commercial production in the United States (US) during 1994. The GM papaya approved for market is resistant to ring spot virus infections and exhibit increased productivity. Today, 80 per cent of Hawaiian papaya grown now is genetically altered, with no other selection available (Bawa and Anilakumar, 2013). The ability to obtain fruit tree plants with new traits or mutations through genetic engineering or NBTs is frequently dependent on availability of a widely recognised *in vitro* regeneration protocol which depends on the genotype and type of beginning plant tissue used (Wang *et al*., 2011; Rai and Shekhawat, 2014; Saporta *et al*., 2017). Several tropical fruits, such as Banana, Citrus, Avocado, Dragon Fruit, Papaya, Mango, and Guava, are gaining popularity for integrating omics techniques (Sabbadini, 2021). In this perspective, breeding methods including tissue culture, embryo rescue, polyploidy, *in vitro* culture, mutagenesis, somaclonal variation, molecular markers, transgenics and genome editing are seen as being essential for trait betterment. Molecular markers made another advancement feasible by selection of features for which the phenotypic is difficult to score until late in seedling culture. Hence it helps for shortening the breeding cycle and reducing the costs also. A large number of molecular markers are now accessible due to the rapid development of genetic research and the widespread availability of sequenced genomes as an effect of the cost reductions obtained through second and third generation sequencing techniques.

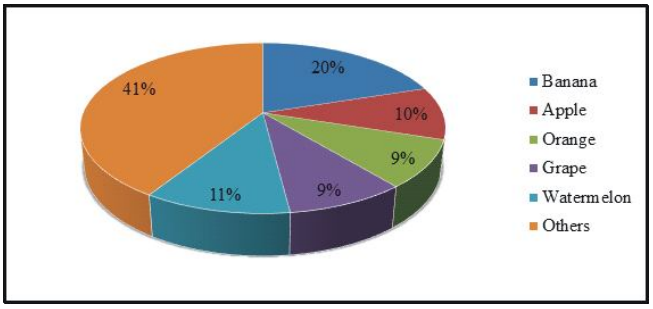


Fig. 1 : Worldwide production of major fruit crop.

New biotechnological tools used for modification of a plant's existing DNA sequence including insertion/ deletion, gene substitution, and stable silence of a gene or promoter region. In this category, we look at RNA interference (RNAi), cisgenesis/intragenesis, trans-grafting and gene editing techniques *viz*.; Zinc finger nucleases (ZFNs) and clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9 system) for introducing new traits into a host plant genome. Although these technologies have been effectively deployed in a variety of crops. Their use in fruit species remains limited. In this context, it is important to enhance the growth and production of genetically elite

fruit species with the approaches, including mutagenesis, *in vitro* culture, molecular markers, transgenics, and genomic breeding tools.

This article provides an overview of recent biotechnological techniques for fruit enhancement. With ongoing advancements, the potential for developing genetically superior fruit species to meet global food demands is immense.

Biotechnological tool in fruit crop improvement

In vitro Multiplication techniques in fruit species

Fruit species such as Peach, Apple, Cherry, Apricot, Citrus, Mango, Banana and Date palm can now be commercially produced using optimized *in vitro* multiplication techniques. *In vitro* technologies have also facilitated the generation of virus-free plants, rapid multiplication of elite clones, somatic embryogenesis, somaclonal variation, transgenic plants, and germplasm preservation. German botanist Gottlieb Haberlandt in the 1890s, pioneered of plant tissue culture (PTC) which has widely used method for rapid asexual reproduction *in vitro*. This technology not only minimizes time and space but also produces superior and disease-free propagules.

Plant tissue culture facilitates micropropagation of several fruit and horticultural crops. including strawberries, papaya, banana, grapes, pineapple and citrus (García *et al*., 2010). Notably, tissue culture-based shoot tip culture techniques have been instrumental in commercial crop micro-propagation, such as in bananas, leading to large-scale *in vitro* multiplication and development of superior planting material. Bananas are produced commercially using tissue culture techniques. The country's banana sector entirely depends on BBTV disease management. Mass propagation by somatic embryogenesis and embryogenic cell suspension (ECS) had been accomplished in the past ten years (Kumaravel *et al*., 2020; Gosh *et al*., 2009) indicating that such techniques may be advantageous to the micro-propagation sector. Using meristem culture, several horticultural crops are now capable of producing virus-free planting materials. Strawberry may be the first fruit crop for which micro-propagation method has been standardised (Sharma and Singh, 1999). Variation of any sort, particularly genetic variants may be viewed obstructive and useless from the perspective of commercial micro-propagation as such variances might result in genetic fidelity being lost.

Embryo Rescue in Fruit Breeding

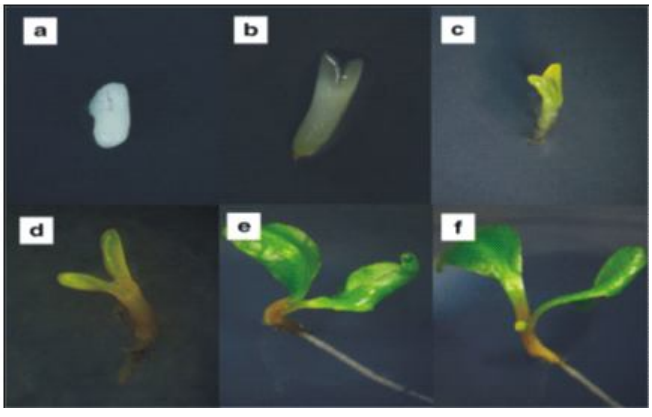
Embryo rescue is another crucial technique in fruit breeding especially for overcoming post-zygotic incompatibility. By culturing excised embryos at appropriate developmental stages, breeders can save crosses from abortion, which is particularly important in recalcitrant and long-lived horticultural plants. Since the early 1980s, embryo rescue has been pivotal in developing new seedless grape cultivars (Ramming and Emershad, 1982). This technique has been widely adopted by grape breeders for producing new seedless varieties by leveraging seedless female parents and embryo rescue procedures. Several grape breeders have attempted to develop new seedless varieties by using embryo rescue technique (Ponce *et al*., 2000; Bharathy *et al*., 2005; Tian *et al*., 2008; Tang *et al*., 2009; Singh *et al*., 2011).

Thompson Seedless and Monukka (both *Vitis vinifera*) are decades old seedless grapes, with the earlier variety being the only known source of seedlessness in existing stenospermic grapes (Stout, 1936; Loomis and Weinberger, 1979; Bouquet and Danglot, 1996). Many breeders have developed new grape cultivars with good fruit quality and production while preserving minor seed traces by using seedless female parents and embryo rescue procedures. Zhiqian *et al.* (2015) used F_1 strains with minimal seed traces from Delight × Ruby Seedless (DRs) as female parents to cross with Thompson Seedless and Monukka to create novel seedless grape germplasm. Progress of DR6 × Thompson Seedless embryo germination represented in Fig. 2.

Somaclonal variation

Somaclonal variation (Larkin and Scowcroft, 1988) is thought to be a source of novel plant genotypes for

Ovules of seedless and seed-trace parents. (a) DR1 ovule with no endosperm; (b) DR1 ovule with endosperm (endosperm marked with arrow); (c) Blush Seedless ovule with no endosperm.



(a) Day of inoculation (0 d); (b) 3 days after inoculation; (c) 5 days after inoculation; (d) 7 days after inoculation; (e) 9 days after inoculation; (f) 11 days after inoculation.

Fig. 2 : Progress of DR6 × Thompson Seedless embryo germination.

breeding, and developments in tissue culture have expanded the potential for viticulture applications. Somaclonal variations have grown widespread and they are now considered a unique method of creating genetic diversity for desirable features. Fruit crops are very susceptible to somaclonal variation because they are mostly vegetatively propagated and have additional breeding difficulties, such as a limited genetic basis and an extended juvenile period. This variation, resulting from epigenetic changes or gene mutations, has been successfully used for *in vitro* selection, leading to the development of superior varieties in fruit crop breeding.

According to Anuradha *et al*. (2017), there is a disadvantage to both germplasm preservation and *in vitro* cloning when insignificant somaclonal variation occurs. The *in vitro* approach for screening desired features has been somaclonal variation followed by *in vitro* selection; the resulting soma clones produced as better varieties have been implemented in fruit crop breeding (Jain, 2001 and Rai *et al*., 2011). A number of novel cultivars were developed for many different kinds of desired characteristics (Table 1). *Giménez et al.* (2001) proposed that in the case of bananas, selection pressure during *in vitro* selection and accurate identification of somaclonal variation can be helpful in incorporating novel characteristics such resistance to yellow Sigatoka, lowering height, and flowering early in order to develop lines for use in banana breeding.

Induced Mutation in fruit crops

The study of mutagenesis has advanced significantly in recent decades to create improved plant cultivars in a variety of agricultural plants, starting with the discovery of X-ray-induced mutations and the development of the first mutants in tobacco and apple (Jain, 2005). The development of desirable mutants that have been approved for cultivation as new crop varieties in a number of countries worldwide has been greatly enhanced by induced mutations (Suprasanna *et al*., 2015 and Sarsu, 2020). Mutagenesis, involving agents *viz.*; EMS and gamma radiation has produced beneficial mutants in various fruit crops, including Orange, Banana, Grape, Peach, Pear, Mandarin and Apple. A notable instance of *in vitro* mutagenesis involved the use of shoot tips in the Grand Nain variety of banana explants; the mutagen source and dose were 80 Gy gamma rays, which resulted in the development of the improved cultivar Novaria-10 with improved traits *i.e.*; early flowering and high keeping quality (Mac *et al*., 1996). While the mutant gamma rays also improved pineapple heat tolerance (Lokko and Amoatey, 2001), fruit colour of apple, dwarf stature of papaya, bunch size and early growth of banana, pear and strawberry disease resistance, and early growth in grapevine (Sattar *et al*., 2021). One promising method for producing a unique genetic resource is through induced mutations. Asexual or vegetatively propagated horticultural plant species may benefit from chimaera separation for fruit-related traits like size, maturity, ripening, colour, self-incompatibility, postharvest quality, and resistance to pests, insects and other diseases through adventitious shoot multiplication or plant regeneration from somatic cells (Geier, 2012).

Transgenic fruit breeding

Fruit crop breeding is hampered by issues such as extended life cycles, propagation techniques, high heterozygosity, and reproductive obstacles; transgenic breeding can help enhance fruit crops in these areas (Gantait *et al*., 2022). Fruit crops have benefited greatly from genetic modification in terms of improved disease

Table 1 : Examples of somaclonal (genetic) variability and development of new.

Fruit cultivar/crop	Resistant varieties	Resistant/ Tolerant to / Improved trait	References
Citrus limon	FS 01 and FS 11	Mal Secco	Gentile *et al.* (1998)
Prunus persica	S-156 (Sunhigh) S122- 1 (Redhaven)	Bacterial leaf spot	Hammerschlag *et al.* (1994)
Citrus sinensis	OLL-4, OLL-8, Valquarius, SF14W-62, UF 111-24	Better yield and fruit quality	Germana *et al.* (2020)
Ananas comosus L. (Merr.)	Cvs. P3R5 and Dwarf	Variation in fruit color, growth habit, fruit size and length of plant generation cycle	Pérez *et al.* (2011)
Blackberry	var. 'Lincoln Logan	Thornless	Hall *et al.* (1986)
Banana cv. William	CIEN BTA-03	Resistance to yellow and black Sigatoka	Unai *et al.* (2004)

Table 2 : Achievements in *in vitro* mutation breeding in vegetatively propagated fruit crops.

Fruit crop	Material use for treatment	Mutagen used (Dose LD_{50})	Plant Regeneration	Result
Banana (*Musa* spp.)	Shoot tips	Carbon-ion beam (0.5-128 Gy)	Direct regeneration	Micro-cuttings from shoots
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	Mutant variety Klue Hom Thong KU1
Pineapple var. Queen	Crowns	γ-rays	Axillary bud regeneration	Lines with reduced spines
Pear	*In vitro* shoots	γ-rays (3.5 Gy)	Micro-cuttings from shoots	Mutants for russeting, fruit shape and size, small tree size, wide branch angle and short internodes

Suprasanna *et al.* (2015)

resistance, drought, cold and salt tolerance, altered plant development patterns, and improved fruit quality (Litz and Padilla, 2012). In 1998, Dr. Dennis Gonsalves and his colleagues in Hawaii, USA, produced and commercialised papaya ring spot virus resistant transgenic papaya (Gonsalves, 1998). SunUp (SunUp x Kapoho). Transgenic papaya cultivars were produced by cloning the CP gene of a mild PRSV strain from Hawaii. In order to determine the fertility and total fruit production of Silcora (s) Def H9iaam lines (GM) and Thompson seedless (TS) in comparison to control Costantini *et al.* (2007). In comparison to the control, the Thompson Seedless DefH9-iaaM line produced twice as many bunches per plant. Sunil *et al.* (2005) analysed transgenic banana plants cultivated *in vitro* and in greenhouses using polyclonal antibody-based ELISA. The genetic modification of pineapple for resistance to the herbicide Basta was studied by Sripaoraya *et al.* (2001). Only when Basta2 concentrations were smaller than 3 mg lÿ1 glufosinate ammonium would non-transformed plants maintain their green colour. Kenong (2013) carried the research to determine how much PPO activity was suppressed in Arctic apples. Transgenic *Prunus domestica* line intron -hair-pin-RNA-B14 from leaves was studied by Hilly *et al.* (2007) in order to identify the plum pox virus 60 days following aphid-mediated inoculation. Transgenic breeding can be a useful method for trait-specific breeding and productivity enhancement (Tanuja *et al.*, 2017).

Molecular marker in Breeding Programs

In breeding programs, molecular markers are valuable for characterising germplasm, boosting the selection of elite alleles at loci controlling critical traits, identifying diversified parents, and protecting intellectual property. Enhancing the production of novel and high-yielding cultivars is the main objective of the breeding program; marker-locus-trait combinations may be utilised as selection criteria for diversified parent selection and selection in population segregation during commercialization. When essential breeding projects get the attention they deserve, marker-assisted breeding can be used to boost program efficiency and save costs. When it came to MAS, the introduction of many markers was essential for quick and effective germplasm assessments, including trait mapping (Abouzari *et al.*, 2020). RAPD was used to identify the female and hermaphrodite sex in papayas (Diwedi *et al.*, 2014) and the same marker was used to identify the male and female in kiwis (Poonam *et al.*, 2001). When it comes to citrus, RAPDs have been used to identify citrus hybrids (Bastianel *et al.*, 1998), characterise cultivars (Deng *et al.*, 2015) build genetic maps (Cai *et al.*, 1994), assess genetic diversity (El-Khayat, 2020) and find markers associated with the cultivars' agronomic aspects (Nicolosi *et al.*, 2000). Li *et al.* (2015) identified seedless parents using a number of markers, including SCC8, SCF27 and GSLP1. Among all the examined markers, only GSLP1 was able to identify the seeded parents.

Genome Editing

The CRISPR system is an advanced adaptive immunity mechanism seen in bacteria and archaea that protects against invading bacteriophages and foreign plasmids (Sternberg *et al.*, 2016). The precise modification of the plant genome is one of the advances

Table 3 : Achievements made in breeding of fruit crops through molecular approaches.

Fruit crop	Marker types used	Work done	References
Mango	AFLPs, RAPDs, SSRs and ISSRs	Identification of hybrids and cultivars	Pandit *et al.* (2007)
Banana	RAPDs, SSRs and ISSRs	Genetic variability and phylogenetic studies	Amorim *et al.* (2009)
Citrus	RFLPs, RAPDs, AFLPs, SSRs, ISSRs, SNPs and DArTs	Identification of hybrids, phylogenetic studies and association of genome mapping to detect various QTLs.	Ahmed *et al.* (2017) and Imai *et al.* (2018)
Grapes	AFLPs, RAPDs, SSRs, ISSRs, SNPs, SCC8, SCF27 and GSLP1	Sex expression, identification of seedless parents, identification of QTLs association with downy mildew resistance	Li *et al.* (2015) and *Divilov et al.* (2018)
Apple	RFLPs, RAPDs, SSRs, ISSRs, SCARs, SNPs and DArTs	Genetic variability, identification of QTLs controlling flesh mealiness, construction of genome map of woolly aphid and detect resistance genes, ACS and ACO, two genes detected for ethylene production by using gene specific markers positioned on a molecular marker linkage map.	Bus *et al.* (2008) and Costa *et al.* (2005)
Guava	RAPDs and SSRs	Genetic diversity and evaluation of genetic variants	Pessanha *et al.* (2011)
Pomegranate	RAPDs, SRAPs, SSRs, ISSRs and SNPs	Genome mapping and genetic relationships	Ophir *et al.* (2014)
Peach	RAPDs, AFLPs, SSRs, SRAPs and SSAPs	Species diversity and identification of brown rot causing genes, *i.e.*, MAT1-1 and MAT1-2	Perez *et al.* (2020) and Rajapakse *et al.* (1995)

of the last ten years (Zhang *et al*., 2021). CRISPR/Cas9, TALENs (Transcription Activator-Like Effector Nucleases) and ZFNs (Zinc Finger Nucleases) are the most widely utilised genome editing tools (molecular scissors). ZFNs and TALENs are the two nucleases that have been successfully edited; however, CRISPR/Cas-9 is a widely used technique that has taken their place. This technique, which involves RNA produced intended nucleases that recognize their corresponding nucleotide sequences in the target sequence (genes), is currently widely used in plant genome editing (Puchta *et al*., 2022 and Kim *et al*., 2017). Many fruit crops, including grape (*Vitis vinifera*) (Jia *et al*., 2017) apple (*Malus domestica*) (Ren *et al*., 2016), grapefruit (*Citrus paradisi*) (Song *et al*., 2013), and sweet cherry (Peng *et al*., 2017) have been subjected to the CRISPR/Cas9 system. Table 4 display several prominent instances of genome editing in fruit crops.

Developing disease resistance against citrus canker in Wanjincheng oranges after the whole EBEPthA4 sequence was deleted from both CsLOB1 alleles is one example of a beneficial outcome (Wang *et al.*, 2018). CRISPR/Cas9-mediated modifications to MdDIPM4 in apples resulted in reduced susceptibility to the fire blight disease pathogen Erwinia amylovora (Tripathi *et al*., 2019), whereas WRKY52 was wiped out in grapes to increase their resistance to Botrytis cinerea (Pompili *et al*., 2020). One of the main issues with ripe bananas is their longer shelf life, which was addressed in (Tripathi *et al*., 2019.) developed bananas by CRISPR/Cas9-mediated targeting of the MA-ACO1 gene. Several studies have shown that genome editing in agricultural plants has the potential to improve global food security and climate resilience (Wolter and Puchta, 2019; Buchholze and Frommer, 2022). Although genome editing of fruit species has been successful, there are still a number of problems that require further study, such as the lacking of annotated genomes, large genome sizes, lengthy *in vitro* growth periods, dependency on a particular genotype and transformation mode, inability to transform a number of fruit crops in a stable manner, and challenges related to polyploidy—that is, having multiple homologous genes. In addition to further exploring CRISPR technology to maximise benefits while

Table 4 : Successful examples of genome editing in fruit crops.

Crop	Target genes	Target trait	Reference
Banana	ORF region of virus	Resistant against banana streak virus	Tripathi *et al.* (2019)
Grape	VvWRKY52	Disease resistance against *Botrytis cinerea*	Pompili *et al.* (2020)
Grape	WRKY52	Resistant to gray mold disease	Wang *et al.* (2018)
Papaya	alEPIC8	Resistant. to *Phytopthora palmivora*	Gumtow *et al.* (2018)
Citrus	LOB1 promoter	Resistant to citrus canker	Peng *et al.* (2017)
Apple	DIPM 1, 2, 4	Resistant to fire blight disease	Malnoy *et al.* (2016)

minimising hazards we must consider public acceptance (Tian *et al*., 2019).

Conclusion

Fruit crop breeding is now experiencing a revolution because of the advancement of biotechnological tools, which have significantly enhanced plant propagation, genetic diversity, and trait enhancement. It has been possible to create superior fruit cultivars with increased disease resistance, better quality, and more adaptability through the use of somaclonal variety, enhanced *in vitro* multiplication techniques, embryo rescue and induced mutations. The advent of transgenic breeding, molecular markers, and genome editing, notably via CRISPR/Cas9, has increased the potential for precise genetic modifications and accelerated breeding processes. Unlike traditional transgenic approaches, CRISPR/Cas9 allows for targeted modifications of the plant's genome, enabling precise edits without introducing foreign DNA. This precision is especially valuable for editing genes associated with important traits, such as disease resistance, drought tolerance or fruit ripening. Furthermore, CRISPR-based breeding could potentially circumvent some of the regulatory barriers associated with genetically modified organisms (GMOs), as the resulting plants are often indistinguishable from those produced through conventional breeding. While challenges remain such as large genome sizes and need for stable transformation methods, ongoing research and technological advancements continue to drive progress in the field, promising even greater innovations and efficiencies in future fruit crop breeding endeavours.

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