horticulturae

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Review

Fruit Crop Improvement with Genome Editing, In Vitro and Transgenic Approaches

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Abstract: Fruit species contribute to nutritional and health security by providing micronutrients, antioxidants, and bioactive phytoconstituents, and hence fruit-based products are becoming functional foods presently and for the future. Although conventional breeding methods have yielded improved varieties having fruit quality, aroma, antioxidants, yield, and nutritional traits, the threat of climate change and need for improvement in several other traits such as biotic and abiotic stress tolerance and higher nutritional quality has demanded complementary novel strategies. Biotechnological research in fruit crops has offered immense scope for large-scale multiplication of elite clones, in vitro, mutagenesis, and genetic transformation. Advanced molecular methods, such as genome-wide association studies (GWAS), QTLomics, genomic selection for the development of novel germplasm having functional traits for agronomic and nutritional quality, and enrichment of bioactive constituents through metabolic pathway engineering and development of novel products, are now paving the way for trait-based improvement for developing genetically superior varieties in fruit plant species for enhanced nutritional quality and agronomic performance. In this article, we highlight the applications of in vitro and molecular breeding approaches for use in fruit breeding.

Keywords: fruits; biotechnological research; induced mutations; genomics; fruit quality; QTLs; transgenic crops; genome editing

Citation: Penna, S.; Jain, S.M. Fruit Crop Improvement with Genome Editing, In Vitro and Transgenic Approaches. *Horticulturae* **2023**, *9*, 58. https://doi.org/10.3390/horticulturae9010058

Academic Editor: Jia-Long Yao

Received: 18 October 2022
Revised: 14 December 2022
Accepted: 19 December 2022
Published: 3 January 2023

1. Introduction

Fruits constitute an important part of human life due to their edible, medicinal and cultural value. Worldwide fruit production has been significant, at 896.45 million tons, with the major proportion contributed by five fruit plants (bananas and plantains, watermelons, grapes, oranges, and apples [1]. Among the fruits, there has been significant production in bananas and plantains (Figure 1). Being natural sources of nutrients and secondary metabolites, fruits are a vital part of the human diet and have nutritional and medicinal properties [2]. Recently, some fruits have assumed the role of functional foods, as they provide antioxidants and medicinal phytochemicals [3,4]. There is a continued demand for fresh fruits and fruit-based products, and hence fruit production of several fruit species has gained momentum and there is a greater focus of breeders to achieve higher productivity.

Post-harvest losses due to perishability of the produce, faster fruit ripening, and loss of nutritional quality have become constraints for achieving higher production and sustained economic gains. In addition, problems such as a long juvenile phase, lower fruit quality, higher numbers of seeds, and incompatibility of rootstock/scion also pose challenges that require continued breeding efforts [5,6]. In this context, conventional breeding methods have significantly contributed to the development of new improved varieties for fruit quality, aroma, antioxidants, yield, and nutritional traits [5]. However, in the wake of climate change and nutritional security, several other fruit crop related traits such as biotic and abiotic stress tolerance and higher nutritional quality demand intensive research inputs and novel strategies of breeding aimed at crop improvement. Several

tropical fruits, including banana, citrus, avocado, dragon fruit, papaya, mango, and guava are now gaining growing attention for implementing integrated omics strategies [4]. In this context, breeding tools such as polyploidy, in vitro culture, mutagenesis, soma clonal variation, molecular markers, transgenics, and genome editing are considered important for trait improvement.

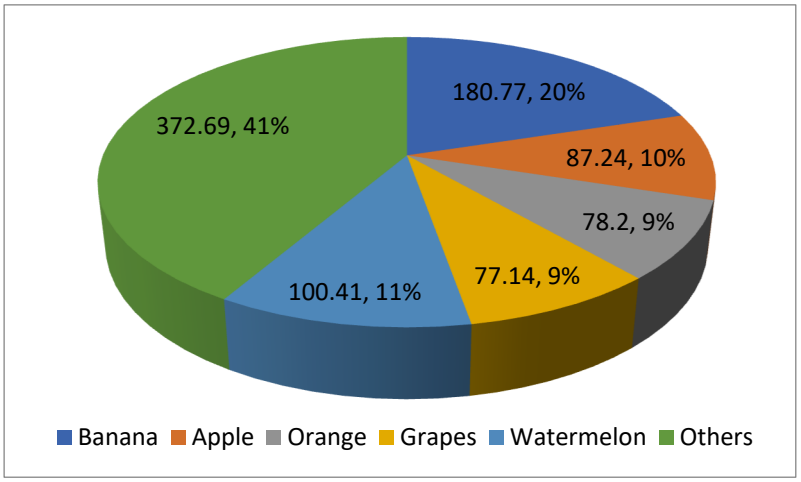

Figure 1. Worldwide production of major fruit crops.

Polyploidy in crop plants is often associated with increased cell size (the gigas effect), and the phenomenon has been exploited in ornamental species [7,8]. Polyploidy has played a significant role in higher heterozygosity, development of novel germplasm, and increased vigor [9]. Polyploid plants also often have novel biochemical, physiological, morphological, and ecological traits for environmental adaptation [10]. Several examples of polyploid fruit species have been reported for higher quality [11], fruit size [12], improved resistance to diseases in *Actinidia* sp. [13], and higher productivity [14], in addition to increased biomass, fruit and flower size, pigment content, and secondary metabolite production [15–17].

Induced mutations have played a key role in the development of desirable mutants that have been released for cultivation as new crop varieties in several countries across the globe [18,19]. Some notable examples include, rice, barley, cotton, groundnut, pulses, ornamentals, rapeseed, and Japanese pear. Biotechnological research in fruit crops—mainly banana, strawberry, papaya, pineapple, apple, citrus, and grapes—has progressed well over other perennial fruit trees. Studies in plant cell and tissue culture have contributed to the development of protocols for large-scale multiplication of elite clones and generation of virus-free planting material, in addition to providing know-how on soma clonal variation, somatic embryogenesis, and genetic transformation. Molecular markers have facilitated the selection of elite clones at early stages of development [20]. The advent of genomics approaches such as genome-wide association studies (GWAS) and QTLomics has created opportunities for molecular breeding [21]. Use of In vitro, mutagenesis, transgenic, and advanced molecular methods has been fundamental to the development of novel germplasm and for generating knowledge about the regulation of functional traits for agronomic and nutritional quality and enrichment of bioactive constituents through metabolic pathway engineering and development of novel products (Figure 2).

The discovery of targeted mutagenesis using genome editing has innovated plant breeding for fine tuning the traits associated with nutritional, floral, and stress tolerance traits in fruit crops such as banana, apple, grapefruit, kiwifruit, and strawberry [22]. Compared to conventional and transgenic breeding methods, genome editing has the advantage of having edited gene(s) for a given trait in a comparatively lesser time span (Figure 3). In other fruits crops, such as jackfruit, guava, and custard apple, which are underutilized, traits such as changing crop habit, crop phenology, and other physiological traits make these fruit trees adaptable to diverse cropping and postharvest patterns, and, hence, considerable

scope exists for bringing these into mainstream cultivations to widen their commercialization and application [23]. In this context, it is desirable to improve the development and production of genetically elite fruit species using strategies ranging from polyploidy to mutagenesis, in vitro culture, molecular markers, transgenics, and genomics breeding tools. In this article, we present an overview on the aspects of fruit improvement using in vitro and molecular breeding approaches.

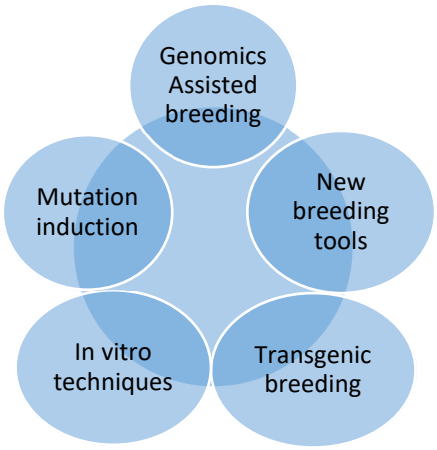

Figure 2. Key research areas for potential interest for fruit crops improvement.

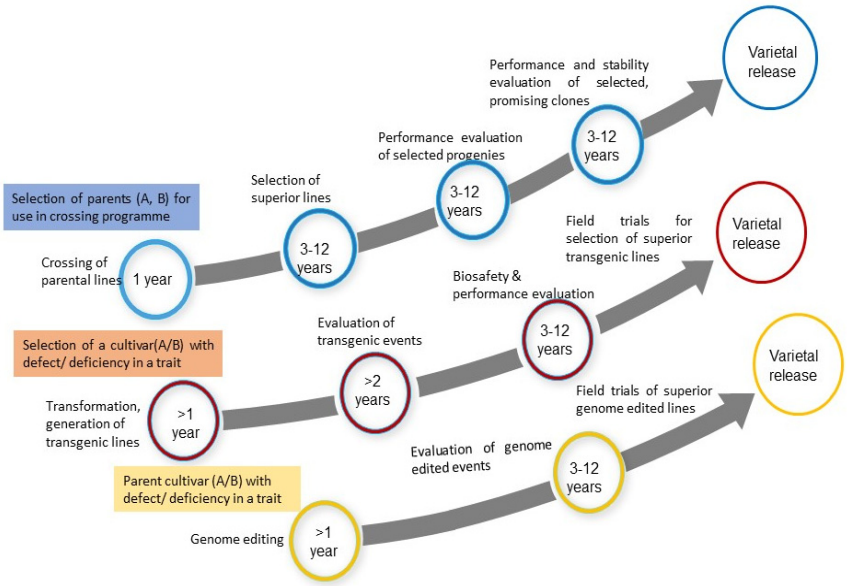

Figure 3. Comparison of conventional breeding, transgenic, and genome editing methods in fruit species [24].

2. Induced Mutagenesis

Since the discovery of X-ray induced mutations and the first mutants developed in tobacco and apple, the field of mutagenesis has expanded tremendously in the past several decades for developing superior plant cultivars in several crop plants [25]. Spontaneous mutation frequency is very low, on the order of one in a million. Both physical and chemical mutagens have been used to enhance the mutation rate by several folds and increase genetic variability in crop plants for a wide range of traits, including yield, plant stature, flowering, salt/drought/heat stress tolerance, disease resistance, high yield, plant architecture, and nutritional quality. Gamma radiation, fast neutrons, and chemical mutagens have been observed to induce mutations such as single-nucleotide alterations, large deletions, and chromosomal aberrations [26]. Alkylating agents such as ethyl methane sulfonate (EMS) are

widely used in fruit crops [27–30]. Globally, several improved mutant cultivars have been released for cultivation in cereals, oil seeds, legumes, medicinal plants, horticultural crops, and plantation crops [31–33]. In the fruits category, 81 mutant varieties have been released for cultivation (Figure 4) [34]. Induced mutations provide a viable option for the generation of a novel genetic resource. In the case of horticultural plant species that are asexually or vegetatively propagated, chimera separation through adventitious shoot multiplication or plant regeneration from somatic cells can facilitate the genetic purity of the mutants [35] for fruit related traits such as size, maturity, ripening, color, self-incompatibility, post-harvest quality, and resistance to insect, pests, and other pathogens. The integrated use of mutagenesis, in vitro culture, and other genomics techniques has further facilitated trait-based improvement in fruit crops [36].

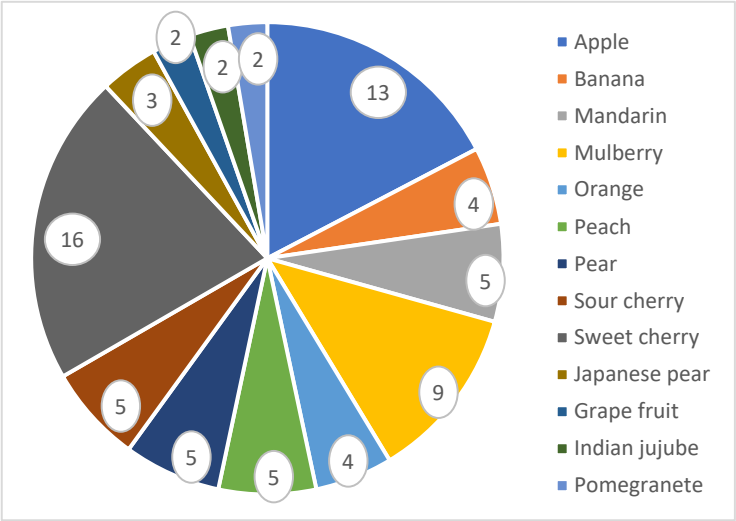

Figure 4. Development of mutant varieties in different fruit species.

Chemical mutagenesis is efficient in whole plants and seeds but less efficient in tissue culture due to toxicity [37]. In banana, mutants for several traits, including reduced height, tolerance to Fusarium wilt, early flowering, large fruit size, and black Sigatoka tolerant types were isolated [32,38,39], whereas mutants tolerant to Bayoud disease were isolated in date palm [40,41], gamma rays to improve heat tolerance in pineapple [42], self-fertility in sweet cherry, fruit color in apple, bunch size and early growth in banana, dwarf stature in papaya, disease-resistance in pear and strawberry, and early growth in grapevine [36].

Mutagenesis enables the creation of genetic variation, especially in those crops having narrow genetic variability, and identification of casual mutations to facilitate improvement in productivity. The development of mutant resources in fruit crops has opened up new avenues for mutant gene discovery [43] using TILLING [44,45] and several high-throughput next-generation sequencing-based techniques, such as MutMap, MutMap-Gap, MutChrom-Seq, Mut-Ren-Seq, and whole-genome sequencing-based mutation mapping which enable detection of hundreds of mutations in a short period of time [46–48]. The MutMap technique offers rapid uncovering of causative nucleotide changes in mutant populations through whole-genome resequencing of mutant plants resulting from crosses of mutants with the parental line [49] and, in contrast, the MutMap+ technique is based on selfing of an M2 heterozygous individual for sequencing purposes and does not need crossing [50]. MutMap-based approaches have been successfully employed in crop plants [51,52].

3. In Vitro Approaches

Plant cell and tissue culture techniques have played a significant role in the multiplication of commercially important fruit species, conservation of genetic resources, production of bioactive compounds, and genetic modification of desirable traits. Commercial production of fruit species has been possible based on optimized protocols of in vitro multiplication

(micropropagation) in peach, apple, cherry, apricot, citrus spp., mango, banana, and date palm. In vitro technologies have also contributed to the development of methods for raising virus-free plants, rapid multiplication of elite clones, somatic embryogenesis, soma clonal variation, transgenic plants, and germplasm conservation. In the case of vegetatively propagated plants, multicellular meristems are often employed for undertaking in vitro mutagenesis [53,54]; however, occurrence of chimeras and phenotypic instability are limitations. Somatic embryogenesis, temporary immersion, and plant cell cultures have immense potential for in vitro propagation and genetic transformation in fruit trees such as mango, banana, pistachio, apple, papaya, coffee, and date palm. Somatic embryogenesis offers many advantages over organogenesis, including its high multiplication rates, scale-up via bioreactors, and delivery through synthetic seeds, in addition to offering as a suitable target for gene transfer [55]. In banana, successful and high frequency somatic embryogenic systems have been developed for use in raising high frequency, large-scale propagation systems and in mutant development using in vitro mutagenesis [56–58]. Embryogenic cell cultures are also advantageous for obtaining non-chimeric progeny as well as for rapid separation of chimeric sectors. A scheme for successful establishment of embryogenic cell suspension cultures derived from male floral apices in banana is depicted in Figure 5, and the system is now routinely applied in various potential applications, including developing synthetic seeds, mutants, transgenic plants, and genome edited plants [59–63].

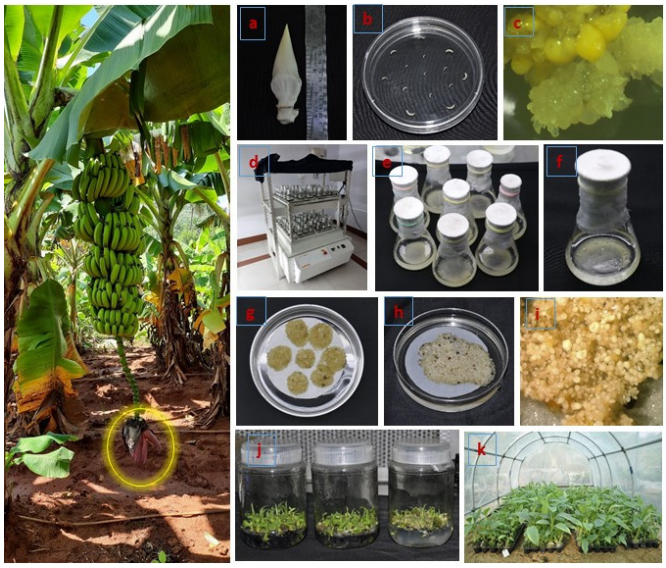

Figure 5. Development of high frequency embryogenic cell suspension cultures in banana (kindly provided by Dr M Saraswathi, NRCB, Trichy, India): (**a**) Explant; (**b**) Initiation of floral hands on callus induction medium; (**c**) Embryogenic calli (microscopic view); (**d**) ECS in orbital shaker; (**e**,**f**) Embryogenic cell suspension; (**g**,**h**) Regeneration or maturation; (**i**) Microscopic view of matured somatic embryos (**j**) Embryo germination; (**k**) Primary hardening.

Plant tissue culture provides immense scope for the micropropagation of fruit and horticultural crops, such as strawberry, papaya, banana, grapes, pineapple, citrus, tomato, cucumber, and watermelon [64,65]. In commercial crops such as banana, tissue culture-based shoot tip culture techniques have played a great role in the micropropagation industry through high volume in vitro multiplication and generation of elite planting material. In the last decade, mass propagation through somatic embryogenesis and embryogenic cell suspension (ECS) has been achieved [66–68], suggesting that such protocols will be of potential benefit to the micropropagation industry. However, the need to maintain genetic uniformity in the clonally propagated plant population has become a major problem for the micropropagation industry, as any genetic variation in the plant progeny is an unwanted phenomenon [69]. The notable example is banana, in which occurrence of off-types from

tissue cultured plantlets ranged from 6 to 38% in Cavendish cultivars [70]; however, it could be as high as 90% as reported earlier [71]. From the point of commercial micropropagation, variation of any kind, in particular, genetic variations may be considered obstructive and worthless, as such variations may lead to loss of genetic fidelity.

Over the past few decades, the occurrence of genetic variations in the in vitro cultured tissues such as undifferentiated cells, isolated protoplasts, and calli tissues have been in evidence [72–74]. Larkin and Scowcroft used the term "soma clonal variation" for variation arising from cell or tissue cultures. Instances of soma clonal variations have become a common occurrence, and presently such variation has become a novel source of inducing genetic variation for desirable traits. Soma clonal variation has tremendous scope in the case of fruit crops, as these are mostly vegetatively propagated and have other breeding concerns such as narrow genetic base and a prolonged juvenile phase. Several new cultivars have been developed for a wide range of desirable traits (Table 1). Soma clonal variation followed by in vitro selection has been used as the in vitro system for the screening of desirable characters in vitro, and the resultant soma clones developed as improved varieties have been used in fruit crop breeding [75,76]. In strawberry, Yoo et al. [77] suggested that selection pressure during in vitro selection and precise detection of soma clonal variation can be useful to incorporate new traits such as resistance to Phytophthora, herbicide tolerance, and heat tolerance to develop lines for use in strawberry breeding.

Table 1. Some examples of soma clonal (genetic) variability and development of new varieties in different plants [78].

Plant	Released Variety	Improved Traits	Reference
Banana	TC1-229	Semi-dwarf and resistant to Fusarium wilt	[79]
	TC2-425	Larger bunch size, resistant to *Fusarium oxysporum* f. sp. cubense (Foc) race 4; high yield	[80]
	CIEN-BTA-03,	Resistant to yellow Sigatoka	[81]
	CUDBT-B1	Reduced height and early flowering	[82]
	Tai-Chiao No. 5	Superior horticultural traits and resistance to Fusarium wilt	[83]
Blackberry	var. 'Lincoln Logan'	Thornless	[84]
Sweet orange (*Citrus sinensis*)	DG-2	Tolerant to citrus canker disease	[85]
Sweet orange (*Citrus sinensis*)	EV1, EV2, N7-3, N13-32, OLL-4, OLL-8, Valquarius, SF14W-62, UF 111-24	Better yield and fruit quality	[86]
Pineapple (*Ananas comosus* L., Merr.)	Cvs. P3R5 and Dwarf,	Variation in fruit color, growth habit, fruit size, and length of plant generation cycle	[87,88]
Tomato (*Lycopersicon esculentum* L.)	DNAP9	High solid contents	[89]

4. Genomics Insights into Fruit Quality

Since the advent of genomics tools and availability of sequencing platforms for most organisms, functional genomic analysis has become a reality in many crops. In fruit crops, genomic sequence information has been reported [24,90,91] paving the way for molecular insights into fruit quality and plant traits based on whole-genome or single gene duplication,

transposon insertions, and gene function. The red fruit color in many fruit crops has been shown to be due to specific MYBs mediated regulation of different anthocyanins, phlobaphenes, and betalains [92]. In addition, MYBs also have a role in carotenoid synthesis and flavor, texture, taste and aroma, astringency, and piquancy [92,93]. It is also possible to induce higher levels of flavonoids through the overexpression approach; for example, in apple, MYB10 resulted in enhanced anthocyanins including epicatechin, procyanidin B2, and quercetin glycosides and, when the MYB10 engineered apples were fed to mice, altered expression of inflammatory genes and reduced inflammation was observed in treated mice [94]. The studies suggest that fruit nutritional quality may be manipulated through MYB transcription factors for better health.

Gene duplications have resulted in fruit sweetness, nutritional content, and quality, e.g., high ascorbic acid associated with *GalUR* gene expansion in orange and jujube fruits [95,96], and fruit sweetness associated with expansion of sorbitol metabolism-related genes *S6PDH*, *SDH,* and *SOT* in fruits of the Rosaceae [97] and pear [98]. Variation in gene structure, phenotype, and functional attributes in fruit crop genomes has been shown to be associated with transposon insertion(s), for example, high-fruit quality in apple with a long terminal repeat retrotransposon insertion upstream of *MdMYB1* [99,100], parthenocarpic fruit development in apple due to insertion in *MdPI* gene [101], TE insertion in the 5′ flanking region of *MYBA1* blocking anthocyanin expression and yielding white berry skin color [102,103], blood orange color due to a *Copia* retrotransposon insertion [104], retrotransposon Rider insertion leading to elongated fruit shape in tomato [105], and transposon insertion in the *YUCCA* gene in peach causing stony hard phenotype [106].

Genomics based approaches have relevance to fruit crop breeding because of the limitations of long generation time and extended juvenile period [24,107–109]. Approaches such as GWAS (genome wide association) enable the appraisal of the positions and effects of QTLs/genes using available cultivars/lines and does not need a segregating progeny [109], whereas genomic selection (GS) can be useful to accurately exercise selection and genetic gain required for the improvement of fruit quality and yield traits [110]. Early selection during the juvenile phase can fasten the selection efficiency to minimize the population required to be taken to subsequent field trials [111,112]. QTLs for several important agronomic traits such as fruit quality and disease resistance have been reported and are being used in marker-assisted breeding [108]. QTLs have been developed for different fruit related characters such as harvest time, fruit skin color, fruit weight, and sugar content in apple, pear, peach, mango, avocado, papaya, and grapevine [113–120]. QTLs for disease resistance include scab resistance in apple [121] and pear [122], plum pox virus resistance in apricot [123], brown rot resistance in peach [124], and downy and powdery mildew resistance in grapevine [125,126]. Major QTLs were mapped on linkage group 3 for skin and flesh color (anthocyanin pigment) in sweet cherry, anther color in almond × peach progenies, and skin color in peach, Japanese plum, and apricot [127,128]. Advances in sequencing methods have facilitated the detection of polymorphism at single nucleotide level in apple, pear [129], prunus [130], plum [131], citrus, and banana [132]. Cao et al. [133] performed GWAS in 104 landrace accessions of peach using 53 genome-wide SSR markers and found good association with fruit traits and phenological period. Another GWAS based study in Japanese pear using 76 cultivars showed association of 162 markers with fruit harvest time and black spot resistance [134]. In a significant GWAS study in apple, Kumar et al. [135] found few hundred SNPs associated with fruit related traits.

5. Transgenic Approaches

Genetic engineering based on genetic transformation methods is now routinely applied to improve plants [136]. Transgenic breeding has an important role in the improvement of fruit crops, as fruit crop breeding is limited by problems such as long-life cycle, propagation method, high heterozygosity, and reproductive barriers [137]. Among the different transformation methods, *Agrobacterium tumefaciens* mediation is widely used based on efficient tissue and cell culture conditions, somatic embryogenesis, and plant regeneration. Genetic

transformation of fruit crops has been very successful for enhancing disease resistance and drought, frost, and salt tolerance, modified plant growth pattern, and fruit quality [6]. Since the first commercialization of genetically engineered Flavr Savr™ tomato [138], several fruit crops have been transformed using a wide variety of genes for improving plant productivity, resistance to insect/pests and diseases, and fruit ripening. There have been several examples of induction of abiotic stress tolerance in fruit crops [5]. Cold tolerance has been developed in apple by overexpression of the cold-inducible Osmyb4 gene [139], multiple stress tolerance in banana plants by overexpressing the stress-responsive WRKY transcription factor (MusaWRKY71) gene [140], drought and salt tolerance in banana through overexpression of the dehydrin gene [141], cold tolerance in papaya by expression of a Transcriptional activator gene, C-repeat binding factor (CBF) [142], and salt tolerance in kiwi fruit through expression of AtNHX1 with high K to Na ratio [143]. To impart resistance to *Xanthomonas* wilt caused by *Xanthomonas campestris* pv. Musacearum, hypersensitive response-assisting protein (Hrap) or plant ferredoxin-like protein (Pflp) gene from sweet pepper (*Capsicum annuum*) was constitutively expressed, and resistance was achieved in banana transgenic plants [144]. Transgenic banana plants of cv. Grand Naine were developed for fungal disease resistance using three genes (endochitinase gene from *Trichoderma harzianum*, stilbene synthase from grape, and superoxide dismutase from tomato) [145]. One of the successful transgenic events in banana include the biofortification of pro-vitamin A by using phytoene synthase enzyme (PSY) and iron (Ferritin gene from soybean) [146,147].

Development of transgenic fruits began with the 'first phase' in which fruit crops such as apple, pear, plum, cherry stock, grapes, walnuts, kiwifruit, citrus, and European chestnut were all transformed using the Agrobacterium method. In the second phase of development, RNAi technologies were majorly adopted for generating GM fruit crops (plum, cherry, apple) in addition to fine-tuning of protocols for Agrobacterium genetic transformation (blueberry, sour cherry), marker-free plants (apple, citrus, and apricot), and commercialization of some transgenic events, such as of non-browning apples. Phase II has covered the development of protocols for genome editing in fruit crops (apple, grape, sweet orange, grapefruit, kiwifruit). In major food crops, adoption of GM varieties has been phenomenal, with large areas under cultivation and economic gain [148]. A report by ISAAA in 2019 estimates that GM crops are grown in 29 countries on 190.4 million hectares (~112-fold increase over 1.7 million hectares in 1996). This includes growing of GM fruit crops in countries such as the USA (papaya, squash, apple), China (papaya), and Costa Rica (pineapple). GM virus-resistant papaya has been the most widely cultivated genetically engineered fruit, followed by virus-resistant squash, apples, and pineapple.

6. New Breeding Techniques

The advances in the new breeding techniques such as cisgenesis/intragenesis, RNAi, and genome editing have provided great impetus to targeted trait improvement [149,150]. It is significant to note the success achieved in the improvement in quality attributes and those conferring better plant architecture and tolerance to biotic and abiotic stresses. The cisgenesis and intragenesis strategies rely on the incorporation of genetic sequences derived from sexually compatible species or the host plant itself (thus obviating the concern of foreign sequences in the host genome), and there have been developments in the isolation and application of cisgenic/intragenic reporter genes and promoters and selectable markers [151,152]. On the other hand, the phenomenon of RNA interference (RNAi) is based on the naturally conserved mechanism in plants and operates through the interference of translation of mRNA through the mediation of double-stranded RNA (dsRNA) molecules that target the silencing of specific transcripts in a sequence-dependent manner [153]. These biotechnological advances have been successfully exploited in fruit crops; however, limitations of efficient regeneration system, long generation time, and genotypic dependency still exist, warranting optimization of culture parameters [154,155]. Nevertheless, there has been significant research efforts in fruit crops on the implementation of the above

NBTs in fruit crop improvement [156], and it is interesting to note that cisgenesis and RNA interference have been successful for the development of transgenic fruit crops for different plant traits (Table 2).

Table 2. Development of genetically engineered fruit crops for functional traits [139].

Improved Trait	Gene	Method	Achievement	References
		Apple		
Resistance to Apple scab (*Venturia inaequalis*)	HcrVf2	Cisgenesis	Plant exhibited reduction in fungal infection	[157–159]
Resistance to Apple scab (*Venturia inaequalis*)	Rvi6	Cisgenesis	Plants had similar resistance to the *M. floribunda* control	[160]
Resistance to Apple scab (*Venturia inaequalis*) strain 104 (Race 1)	Rvi6	Cisgenesis	Plants showed resistance to *Venturia inaequalis* strain 104 (Race 1)	[161]
Resistance to Apple Rvi6 scab	HcrVf2	Cisgenesis	Cisgenic lines containing the HcrVf2gene	[162]
Resistance to fire blight (*Erwinia amylovora*)	FB_MR5	Cisgenesis	Plants expressed lower disease symptoms	[163]
Resistance to powdery mildew (*Podosphaera leucotricha*)	MdMLO19	RNA interference	Transgenic apple lines resistant to powdery mildew	[164]
Resistance to crown gall formation	iaaM and ipt	RNA interference	Transgenic apple lines resistant to crown gall formation on tree roots	[165]
Early flowering induction	MdTFL1	RNA interference	Silencing of PcTFL1-1 and PcTFL1-2 genes in transgenic pear with consequent early flowering phenotype	[166]
Dwarf plant type	MdGA20-ox	RNA interference	Transgenic apple lines with reduced height, shorter internode length, and higher number of nodes	[167]
The reduction of fertility and the increase of floral attractiveness	MdAG-like genes: MdMADS15 and MdMADS22	RNA interference	Trees with polypetalous flowers. Reduced male and female fertility of flowers	[168]
Improve post-harvest fruit quality	Endo-polygalacturonase1 PG1)	RNA interference	Increased post-harvest fruit quality	[169]
Grapevine (*Vitis vinifera* L.): Resistance to *Powdery mildew* (*Erysiphe necator*)	VVTL-1	Cisgenesis	Plants showed delayed disease development and decreased severity of black rot (*Guignardia bidwellii*)	[170]
Papaya (*Carica papaya*): *Papaya ringspot* virus (PRSV)	PRSV-CP	RNA interference	Resistance to PRSV *Transgenic papaya* resistant to *Papaya ringspot* virus (PRSV)	[171,172]
Plum (*Prunus domestica* L.): plum pox virus (PPV)	PPV-CP		Resistance to Sharka (PPV) *Transgenic plum* clone Honeysweet resistant to sharka disease	[173–175]
Sweet orange (*Citrus sinensis*): *Citrus psorosis* virus (CPsV)	CPsV-CP	RNA interference	Resistance to CPsV Transgenic sweet orange plants resistant to CPsV	[176]
Grapefruit (*Citrus paradisi*): *Citrus tristeza* virus (CTV)	CTV	RNA interference	Resistance to CTV Transgenic grapefruit lines resistant to CTV	[177]

7. Genome Editing

One of the advancements in the past decade has been the precise editing of the plant genome [178]. Plant genome editing has immense scope for the targeted modification of plant genes controlling various important traits. The method is based on a restriction nuclease that can detect specific sequences in genomic locations and subsequently cut the specific gene sequences. Named molecular scissors, the nucleases are zinc finger nucleases (ZFNs), transcription activator-like effector-based nucleases (TALEN), and the clustered regularly interspaced short palindromic repeats associated nucleases (CRISPR/Cas) [179,180]. All these nucleases (ZFNs and TALENs) are studied with successful editing opportunities in a wide variety of organisms ranging from plants to animals; however, based on the cumbersome

methodologies and cost involved in the genome editing process, these are now replaced by a popular method referred to as CRISPR/Cas. Currently, this method is extensively used in plant genome editing [179,181] and comprises RNA guided engineered nucleases that recognize their associated nucleotide sequences in the target sequence (genes). The process of genome editing is accomplished either by directly delivering the single-guide RNA (sgRNA) with pre-complexed purified CAS9 protein or through cell transfection with a plasmid encoding CRISPR-associated protein 9 (CAS9) and sgRNA [182]. CRISPR/Cas9 system has been applied in several fruit crops, including tomato (*Solanum lycopersicum*) [183], apple (*Malus domestica*) [184], grape (*Vitis vinifera*) [185], grapefruit (*Citrus paradisi*) [186], sweet cherry [187], strawberry, orange, and banana. Some notable examples of genome editing in fruit crops are presented in Table 3.

Some successful examples include development of disease resistance against citrus canker in Wanjincheng orange upon the deletion of entire EBEPthA4 sequence from both *CsLOB1* alleles [188], high resistance of grapes to *Botrytis cinerea*, by CRISPR/Cas9-mediated knockout of WRKY52 [189], and lower susceptibility to the fire blight disease pathogen, *Erwinia amylovora*, in apple by CRISPR/Cas9-mediated alteration in *MdDIPM4* [190]. Tripathi et al. [191] developed banana plants resistant to banana streak virus, one of several serious diseases, through CRISPR/Cas9 mediated induction of mutations in the BSV sequences. They observed that genome-edited plants displayed no symptoms of viral disease. Banana plants exhibiting dwarfism have also been generated using the CRISPR/Cas9 system by mutations in the MaGA20ox2 gene, which regulates endogenous GA levels [192,193] suggesting the usefulness of the genome editing tool for developing dwarf banana cultivars.

Table 3. Successful examples of genome editing in fruit crops.

Plant	Method	Target Gene	Trait	Modification	Reference
Citrus	CRISPR/Cas9 (SDN1)	CsLOB1	Disease susceptibility gene for citrus bacterial canker	Mutant plants exhibited improved fungal resistance	[188]
Grapevine (*Vitis vinifera* L.)	CRISPR/Cas9	MLO-7	Resistance to powdery mildew	Efficient targeted mutagenesis	[194]
Grape	CRISPR/Cas9 (SDN1)	VvWRKY52	Disease resistance against *Botrytis cinerea*	Mutants plants showed higher resistance	[189]
Grape	CRISPR/Cas9 (SDN1)	IdnDH	Tartaric acid biosynthetic pathway	High levels of tartaric acid in mutants	[185]
Apple	CRISPR/Cas9	MdPDS	Important enzyme in TA biosynthetic pathway	Albino phenotype in plants	[184,195]
Banana	CRISPR/Cas9	MdDIPM4	Fire blight disease susceptibility protein	Reduced susceptibility to the pathogen, *Erwinia amylovora*	[190]
	CRISPR/Cas9	RAS-PDS genes (RAS-PDS1 and RAS-PDS2)		Complete albino and variegated phenotypein the plantlets	[196]
	CRISPR/Cas9	PDS		100% mutation rate and triallelic deletions or insertions among the plants	[197]
	CRISPR/Cas9	MA-ACO1	A key component of the ethylene biosynthetic pathway	Plants were characterized by extendedshelf-time	[198]
		Musa dmr6	Banana Xanthomonas wilt	Mutants showed enhanced resistance to important disease, BX	[199]

Fruit crop improvement through conventional means is often hampered by a long generation period and long juvenile period, in the range of 13–16 years. Since juvenility is associated with high levels of terminal flowering (TFL) protein, intersecting studies by Charrier et al. [190,200] demonstrated early flowering by editing of the genes (MdTFL1.1, PcTFL1.1), resulting in 93% apple lines and 9% pear lines. Such studies go a long way in

establishing fast breeding schemes for fruit crops. In other interesting research, the juvenile phase in fruit crops could be shortened through inducing a flowering gene or/and silencing a floral repressor combined with MAS [191,192,201,202].

There have been several studies demonstrating the potential of genome editing in crop plants and that the technology could contribute to global food security and climate resilience [203,204]. Globally, several countries have already adopted regulations for the GE plants for commercialization and/or cultivation [205] based on the premise that the use of the GE crops in agriculture is similar to conventionally bred lines, provided they do not contain a transgene. Menz et al. [206] introduced the term 'marker-oriented' for the genome edited events based on the criteria that the editing is applied in a crop plant or ornamental plant, the trait has relevance to plant functionality, and the event is analyzed as being distinct and unique. In an interesting study, Shew et al. [207] investigated the public responses in some countries, including the USA, Canada, Belgium, France, and Australia, to see if they were willing to accept and consume both GM and CRISPR foods. The data suggest that 56, 47, 46, 30, and 51% of respondents in the USA, Canada, Belgium, France, and Australia, respectively, showed willingness to consume both GM and CRISPR foods and were more willing to consume CRISPR than GM food.

Buchholzer and Frommer [205] summarized the present status of GE crops in the global context and concluded that the USA and several other countries were classified as transgene-free, and that they are as equivalent to conventionally bred lines. Several countries, including Russia, countries in Central and South America, two countries in Africa, China, and India have developed and placed new guidelines for the use of genome-edited plants in agriculture (Figure 6). Regulatory guidelines to exempt GE crops from GMO regulations are also being proposed in Europe, the UK, and Switzerland [205].

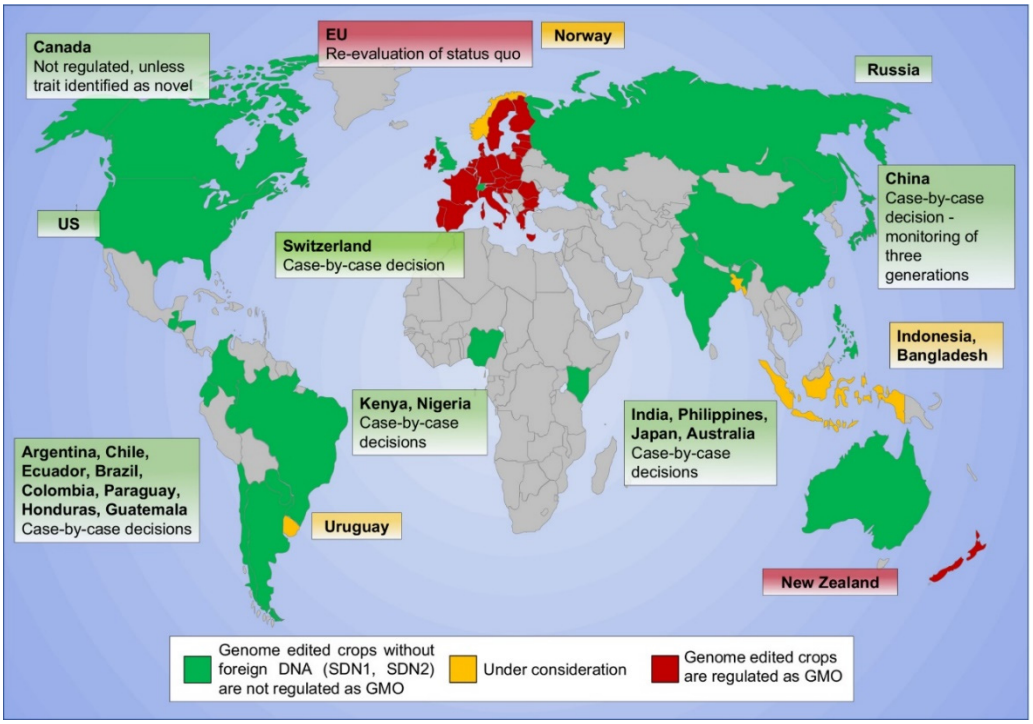

Figure 6. Global state of regulation of genome editing (Adapted with permission from John Wiley Ref. [205]).

Despite the success achieved in genome editing of fruit species, some of the challenges that need extensive research include lack of annotated genomes, large genome size, long in vitro growth periods, dependence on a specific genotype and mode of transformation, lack of stable transformation of a wide range of fruit crops, and polyploidy associated problems of having multiple homologous genes. There has been some success of multiallelic

editing in the case of banana [206] and induction of allelic variants and their segregation to enable developing genome editing in polyploid species [207]. Further, genetic variation in fruit architecture or quality traits governed by SNPs can be manipulated through base replacement using a homology directed repair method [208]. Successful examples include tomato [208,209]. Prime editing has been developed to enable precise alteration of a specific DNA base replacement [210,211].

8. Conclusions

Fruit crops constitute an important part of global food production system and significantly contribute to food and nutritional security. Breeding approaches have played critical roles in crop improvement; however, in the case of fruit crops, being vegetatively propagated and often having long juvenile life, alternative approaches for their improvement are required. In vitro culture technologies have offered the scope for high-volume propagation and multiplication of elite clones, somatic embryogenesis, cell cultures, enhancement of genetic variability through soma clonal variation, and generation of transgenic plants. Success has been achieved in fruit crops such as banana, strawberry, papaya, pineapple, apple, citrus, and grapes. The application of embryogenic cell suspension cultures, as in the case of banana, has transformed the crop improvement approach using mutagenesis, transformation, and genome editing tools. However, fine tuning of in vitro protocols could further boost commercial propagation of elite cultivars in banana and other important fruit crops. Important fruit crops that are polyploid will require new methodological innovations for investigating genetic architecture of fruit related traits such as fruit firmness, ripening, longer shelf life, traits for mechanical harvesting and nutritional traits. Extensive genomics research inputs are warranted for the identification of functional and regulator genes, functional markers associated with plant phenotypic variation, well-defined genetic maps, QTLs, genome-wide association studies for improved fruit quality, and other plant traits. New plant breeding tools including genome editing and cisgenesis have shown promise for developing plant engineered for functional traits such as resistance to diseases in Citrus spp. and nutritional quality in banana, pear, and walnut. Molecular breeding in the coming years will undoubtedly pave the way for production of elite fruit crop varieties to meet food and nutritional security.

Author Contributions: Authors have equally contributed to conceptualization, writing, reviewing and finalization of the manuscript for publication. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Authors acknowledge all the authors whose was described in this manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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