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OBM Genetics

Review

Agrobacterium-Mediated Genome Modification for Improvement of Oil Palm Planting Materials

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Academic Editor: Vishnu D Rajput

Special Issue: Genome Editing for Crop Improvement

OBM Genetics
2025, volume 9, issue 2
doi:10.21926/obm.genet.2502292

Received: December 18, 2024
Accepted: March 30, 2025
Published: April 15, 2025

Abstract

Oil palm is the most productive vegetable oil crop compared to other oil-bearing crops because it produces the highest oil yield per hectare. Palm oil is very versatile since it is used for producing food and beverages, personal care and cosmetics, cleaning products, biofuel, and bioenergy. To cater to the increasing demand in the global palm oil market, much research has been done to improve the oil's yield and modify its quality in addition to the oil palm height through breeding. Due to its long breeding cycle, oil palm planting materials have been improved using biotechnological approaches such as genetic engineering and genome editing. The ability to transform oil palm with high efficiency is the key to effect genome modification of the palm. The current oil palm transformation efficiency for *Agrobacterium*-mediated transformation is very low compared to other monocots such as rice, maize, and wheat. Over the last few decades, numerous studies have been conducted to enhance the transformation efficiency, providing a more reliable landscape for CRISPR/Cas9 genome editing. In this review,

we discussed the progress made in oil palm genetic transformation, focusing on the *Agrobacterium*-mediated transformation, and outline possible strategies to enhance transformation and regeneration efficiencies. The progress and prospect of *Agrobacterium*-mediated CRISPR/Cas9 genome editing for improving oil palms agronomic traits, such as oil yield, plant height, fruit color, and resistance to resist biotic and abiotic stresses, were also discussed.

Keywords
Agrobacterium-mediated transformation; CRISPR/Cas9; transgenic oil palm; oil palm genome editing

1. Introduction

Oil palm (*Elaeis guineensis* Jacq.) is a highly productive vegetable oil crop compared to other crops. The oil yield is about 3-6 tons per hectare, depending on the type of plantations [1]. In 2023, Malaysia's oil palm cultivation area covered 5.65 million hectares [2]. Malaysia ranked second place as the palm oil producer after Indonesia. Other palm oil-producing countries are Thailand, Colombia, Nigeria, and others [3]. In 2022, palm oil contributed to 60% of the global vegetable oil trade as compared with soybean (14%), sunflower (10%), coconut (2%) and olive (1%) [4]. In 2023, Malaysia produced about 18.55 million tons of palm oil, supplying 24% of global palm oil production as compared to 47 million tons (59%) from Indonesia [3]. Malaysia exported 15.13 million tons of palm oil, worth RM 62.58 billion in 2023 [2]. India remains the major importer of Malaysia's palm oil, followed by China, the EU, Kenya, Turkiye, Japan, and Pakistan, with a total export of 8.23 million tons [2].

Palm oil is versatile since it is used in manufacturing food and drinks, personal care and cosmetics, cleaning products, biofuel, and bioenergy. In addition, oil palm biomasses can be used to make furniture, car components, feed formulation, and natural fertilizer. The Organization for Economic Cooperation and Development (OECD) and the Food and Agricultural Organization (FAO) predicted that by the year 2032, the food use of global vegetable oil will hit 57% of total consumption due to increased population growth and increased use per capita especially in the lower- and middle-income countries [5]. For 2024, the United States Department of Agriculture (USDA) predicted that worldwide palm oil consumption might rise by 4.9% to reach 77.2 million metric tons [6]. In order to support the increasing need for palm oil, conventional breeding and biotechnology are employed to improve the oil's yield and quality.

The oil palm is a long-lived tree species with a productive lifespan ranging from 20 to 25 years, and it took nearly 50 years to introduce a new trait into this palm, considering crossing and backcrossing [7]. Significant achievements have been made in improving oil quality and yield as well as other planting material attributes such as height and disease resistance [8]. The Malaysian Palm Oil Board (MPOB) has introduced 14 planting series with traits such as dwarf, high carotene content, high vitamin E, high bunch index, and high oleic acid using germplasm collection [9]. Other target traits that can only be produced through genetic engineering, such as high ricinoleic acid content and biodegradable plastic [10], were also explored. Genetic engineering is beneficial in oil palm

breeding since this technique could reduce time and cost, increase the precision of trait introgression, and widen the genetic background of oil palm [7]. MPOB has embarked on an oil palm genetic engineering program since 1987, focusing on a high oleic acid trait that increases palm oil's marketability for liquid and salad oil consumption [11, 12].

Transgenic oil palms were successfully produced using the biolistic delivery method, *Agrobacterium*-mediated transformation, and DNA microinjection [12]. Nevertheless, the efficiency of transformation was notably low, ranging from 0.7% to 1.5% [13] in comparison with other monocots, such as rice at 14 to 26.4% [14] and sorghum at 14 to 33% [15]. The success of oil palm genome sequencing [16] opens up new opportunities. It paves the way for the oil palm genome editing program using CRISPR/Cas9 technology. In 2021, Yeap and co-workers [17] published the first victory of oil palm genome editing, followed by Bahariah et al. [18], Jamaludin et al. [19], and Norfaezah et al. [20]. Therefore, this review discusses the progress of *Agrobacterium*-mediated transformation and its application in oil palm genetic engineering and genome editing, aiming for a sustainable future of the oil palm industry.

2. *Agrobacterium*-Mediated Plant Transformation

Agrobacterium-mediated transformation is widely utilized as a tool for crop improvement. This system utilizes *Agrobacterium*, a gram-negative soil bacteria, to integrate foreign DNA into the plant genome. The most commonly used *Agrobacterium* is *A. tumefaciens,* which triggers crown gall disease, and *A. rhizogenes,* which triggers hairy root disease naturally in infected plants [21]. The discovery of *Agrobacterium* was announced in 1907 by plant pathologists Erwin Smith and Charles Townsend, who studied the contributing factors to crown gall disease in ornamentals [22]. The history of *Agrobacterium* discovery and the subsequent breakthrough technologies have been extensively reviewed by Nester [22]. For instance, in 1974, Zaenen and co-workers discovered an enormous plasmid during the crown gall formation, and six years later, Zambryski and colleagues demonstrated that the plasmid's fragment was arbitrarily incorporated into the plant's genome [22]. Besides plant cells, this remarkable bacterium was also capable of transferring its T-DNA (transferred DNA) into fungi, algae, and human cells [23].

In dicotyledons, *Agrobacterium*-mediated transformation has always been the method of choice because they are the natural host of the bacteria. Since the elucidation of the basic mechanism of tumorigenesis in crown gall disease, methods to produce tumor-free transgenic plants were established for tobacco (*Nicotiana tabacum* L.) and petunia (*Petunia × hybrida*) [24], soybean (*Glycine max* L.) [25] and other dicotyledons by the middle of 1980s. The first commercialized transgenic dicotyledon crop developed by *Agrobacterium*-mediated transformation was the Flavr Savr™ tomato [26]. *Agrobacterium*-mediated transformation for monocotyledonous plants was initially considered impossible because they are not the natural hosts for *Agrobacterium* and, thus, are not prone to crown gall disease. This paradigm revolutionized in 1987 when Bytebier and co-workers demonstrated that *Agrobacterium* T-DNA could be incorporated into the genome of asparagus (*Asparagus officinalis)*, a monocotyledon plant [27]. The first reported successful transformation of monocot commercial crops was rice (*Oryza sativa*) using the mature embryos and maize (*Zea mays*) using the shoot apex [24]. Subsequently, genetic engineering via *Agrobacterium* has been successfully employed to modify cereal crops such as sorghum (*Sorghum bicolor* L.), barley (*Hordeum vulgare* L.), as well as wheat (*Triticum aestivum* L.) [24]. However, herbicide-tolerant and

insect-resistant maize were the only transgenic cereals that were planted for commercialization on 57.4 million acres globally [24]. In addition to cereal crops, *Agrobacterium*-mediated transformation was also utilized to enhance the quality of woody fruits and nuts [28]. The virus-resistant "Honey Sweet" plum has been granted for planting in the USA, while the non-browning apple has already been marketed for consumption [28]. Besides, *Agrobacterium*-mediated transformation was also employed to improve prominent oil-bearing crops such as oil palm [27, 29-32], soybean (*Glycine max* L.) [25], sunflower (*Helianthus annuus* L.) [33], and rapeseed (*Brassica rapa* and *B. napus*) [34]. The progress made in *Agrobacterium*-mediated transformation for oil palm improvement is listed in Table 1.

Table 1 Application of *Agrobacterium*-mediated oil palm transformation.

Explant	*Agrobacterium* strain	Vector	Selection agent	Physical treatment	Trait	References
EC*	LBA4404	pLYCRISPRCas9P35S-H: EgFAD2 pLYCRISPRCas9PUbi-H: EgPAT pLYCRISPRCas9PUbi-H: EgFAD2EgPAT	Hygromycin	Bombardment	High oleic	[18]
EC*	LBA4404	pUBA	Basta	Bombardment	Basta resistance	[27]
IE*	LBA4404	pCAMBIA1301	Hygromycin	NA*	Hygromycin resistance	[29]
EC*	AGL-1	pCAMBIA1304	Hygromycin	NA*	Hygromycin resistance	[30]
EC*	LBA4404	pBIDOG	2-Deoxyglucose (2-DOG)	Bombardment	2-DOG resistance	[31]
EC*	EHA105	pBINUBG, pPZUBG, pNH1, pNH2, pBAR65, pBARSGFP	Basta	Sonication, vacuum infiltration	Basta resistance	[32]
EC*	AGL-1, EHA101	pCAMBIA1304 pIG121	Hygromycin	NA*	Hygromycin resistance	[35]
IE*	LBA4404	pFA2, pFA3, pMR505	Hygromycin	Cut in half	Bioplastic	[36]
EMB*	AGL-1	pCAMBIA1304	Hygromycin	Sonication	Hygromycin resistance	[37]
IE*	LBA4404	pCAMBIA1302 pCAMBIA1304	Hygromycin	NA*	Hygromycin resistance	[38]
IE*	LBA4404	pJLPHB3	Hygromycin	Cut in half	Bioplastic	[39]
IE*	LBA4404	pRMIN pLMIN	Hygromycin	NA*	Bioplastic	[40]
EMB*	EHA105	pCAMBIA1300-*EgMADS21*	Hygromycin	NA*	High oleic	[41]
EC*	LBA4404	CRISPR-PTE	Kanamycin	NA*	High saturated fatty acid	[42]

* IE Immature embryo; EC Embryogenic calli; EMB Embryoids; NA Data not available.

Agrobacterium-mediated transformation is a more favored transformation method compared to other methods because it is easy, economical, has less incidence of transgene re-arrangement, is capable of delivering large DNA fragments, and is excellent in the integration of transgene into transcriptionally potent domain, which will lead to successful transgene expression in the plant cells [43]. The *Agrobacterium* transfers gene(s) into the plant cells in five crucial stages: (a) Stimulation of *Agrobacterium* virulence mechanism, (b) Formation of T-DNA complex, (c) Movement of T-DNA from *Agrobacterium* to the plant cell nucleus, (d) Integration of T-DNA into the plant genome and (e) Expression of the T-DNA in the plant cells [21]. The fruitful transformation of plants was accomplished by optimizing parameters and developing an efficient protocol where the selected parameters act synergistically during transformation [44]. In many *Agrobacterium*-mediated transformation studies, the transfer of T-DNA and its integration into the plant genome is affected by the plant's genotype, type of explant, transformation vector, *Agrobacterium* strain, composition of the culture medium, co-cultivation temperature, co-cultivation duration, *Agrobacterium* density, selectable markers, chemicals, and surfactant [45-47]. If these factors are sufficiently optimized, it will facilitate the integration of T-DNA into the plant genome, as it impedes successful *Agrobacterium*-mediated transformation in numerous plant species [45].

3. Strategy to Improve *Agrobacterium*-Mediated Transformation Efficiency

Although stable transgene integration in the oil palm genome via *Agrobacterium*-mediated transformation has been demonstrated [27, 31], efforts to improve the transformation efficiency are still being carried out. Compared to other monocots, the oil palm's transformation efficiency is still relatively low. The transformation efficiency was only 0.7% when the bialaphos resistance or *bar* gene was utilized as the selectable marker [27]. Later, the transformation efficiency increased to 1.0% when the 2-deoxyglucose-6-phosphate phosphatase (*DOG*^{R}*1*) gene was used as the selectable marker for oil palm transformation [31]. Currently, the oil palm embryogenic calli [27, 31, 35] and immature embryos [29, 36] are the most widely used explants for *Agrobacterium*-mediated oil palm transformation.

Optimization of other critical parameters for *Agrobacterium*-mediated transformation of oil palm was also carried out. Among the parameters are *Agrobacterium* strains [35], *Agrobacterium* density, inoculation and co-cultivation periods [30], and transformation vectors [32, 36]. Sonication treatment was also evaluated as the wounding method, but the study was done on oil palm somatic embryos rather than embryogenic calli and immature embryos [37].

However, a systematic comparison of other *Agrobacterium*-mediated oil palm transformation parameters, such as explant type and age, acetosyringone concentration, and physical injury methods, has not been reported. Systematic optimization of all critical parameters involved in *Agrobacterium*-mediated transformation could significantly enhance transformation efficiency and regeneration frequency as observed in other plants [13], whether the plant is readily transformable or previously described as recalcitrant to transformation and regeneration via tissue culture.

According to Ziemienowicz [46], *Agrobacterium* concentration, age of callus, wounding methods, preculture periods, co-cultivation periods, and acetosyringone concentrations are among the crucial parameters that require optimization to establish an effective *Agrobacterium*-mediated transformation procedure for any given species. Other factors can also influence *Agrobacterium*-mediated plant transformation, including inoculation time [48], vector plasmid, bacterial strain [49],

and resting periods [50]. According to Li et al. [47], the overall transformation efficiency is influenced by the efficaciousness of *Agrobacterium* infection and explant regeneration. To establish an effective transformation protocol, researchers must determine the precise ratio of many elements that interact during the transformation process [44]. An efficient transformation and regeneration protocol will enable the execution of CRISPR/Cas9 genome editing technology to regenerate transgene-free, stably edited plants with economically important traits, thereby supporting their sustainability in the current bioeconomy era. Studies related to optimizing critical parameters for *Agrobacterium*-mediated transformation are discussed in the following subtopics.

3.1 Agrobacterium Strains and Transformation Vectors

One of the most critical factors in *Agrobacterium*-mediated transformation is the infecting ability of the *Agrobacterium* strain, as each strain has a distinct capacity to transform plant tissues [44]. In general, the nopaline strains have a better potential to infect woody species than the octopine strain [51]. The combination of *Agrobacterium* strains and vectors was known to affect the transformation efficiency of plants [45]. For *japonica* rice transformation, LBA4404 carrying a super-binary vector pTOK233 was more effective than super virulent strain EHA101 carrying the same super-binary vector in transforming Tshukinohikari and Koshihikari cultivars [24]. The EHA105 strain was superior to LBA4404 in maize transformation [52].

Boonyaves et al. [53] showed that EHA105 gave the highest transient expression of the β-glucuronidase (*gus*) gene compared to AGL-1 in oil palm suspension culture. Promchan and Te-chato [35] reported that EHA101 was more efficient than AGL-1 in infecting oil palm suspension culture. In *Jatropha curcas*, EHA105 was superior to LBA4404, GV3101, and AGL-1 in infecting *J. curcas* cotyledon and was able to transfer a single copy of the transgene [54]. These studies were consistent with the survey in Micro-Tom tomato, which defined that *Agrobacterium* strain EHA105 was the most efficient compared to AGL-1 and GV3101 and was able to produce transgenic plants carrying a single copy of transgene [55]. Besides the host strain, Hanin et al. [32] demonstrated that a vector carrying the *bar* and green fluorescent protein (*gfp*) genes controlled by the *CaMV*35*S* promoter in the pBINPLUS/ARS backbone was more efficient than vectors in pCAMBIA0380 backbone based on transient GFP signals. The aforementioned work highlights the importance of evaluating the bacterial host strain and vector components to enhance the success of a transformation event.

3.2 Explant Types and Pre-Treatments

Studies in other crops have shown that *Agrobacterium* infection exhibits a varying affinity for different plant tissues, organs, and cells. Ribas et al. [56] evaluated three types of somatic embryogenic tissues, namely embryogenic calli, established embryogenic calli suspension, and embryogenic callus culture, and concluded that the established embryogenic calli gave the highest transformation efficiency (17%). *Agrobacterium*-mediated transformation was performed for oil palm using immature embryos (IEs) as the explant in the early research due to their abundance [29]. A bunch of oil palm fruits yielded about 300 to 500 IEs. Nevertheless, the genetic composition of IEs is not homogenous since they are the result of cross-pollination [29]. Thereafter, somatic embryogenic suspension cultures (ECs) were chosen as the preferred explant [13]. The oil palm ECs were proposed as the ideal explant for oil palm transformation via *Agrobacterium* owing to their unicellular nature [35]. The use of a single cell could prevent chimerism in the transformed tissue,

thus leading to the regeneration of actual transgenic plants, as reported by Huang et al. [57]. Immature embryogenic calli were also utilized for oil palm transformation [58]. In addition, evaluation of oil palm immature leaves as an explant has also been suggested [59].

In an *Agrobacterium*-mediated transformation system, the explants were wounded to increase transformation efficacy [27]. Wounding triggers the production of phenolic compounds by the injured cells, which are required to activate virulence functions within *Agrobacterium* [27]. Wounding by bombardment was reported to increase transformation efficiency in carnation (*Dianthus caryophyllus* L.) [27]. In addition, sonication treatment, which is more cost-effective than bombardment, was proven to enhance the transformation efficiency of chickpeas, soybeans, and flax [60]. Furthermore, a combination of vacuum infiltration and sonication was proven to increase the transformation efficiency of banana cv. Rasthali [61] and *Whitania somnifera* (L.) Dunal [62]. Therefore, sonication and vacuum infiltration can be potential alternatives to bombardment to increase transformation efficiency in oil palm.

Besides wounding, osmoticum or plasmolysis treatment was used to increase transformation efficiency. Plasmolysis treatment with 10% sucrose prior to *Agrobacterium* increased transient GUS expression in rice calli [27] and citrus [63]. A combination of plasmolysis treatment with 6% sucrose and bombardment prior to *Agrobacterium* infection in oil palm embryogenic calli was demonstrated by Masli et al. [27] and Izawati et al. [31]. However, since the protocol utilizes only one treatment parameter, a combination of 6% sucrose and bombardment, it is essential to reevaluate the necessity of the plasmolysis treatment.

3.3 Transformation Conditions

A review by Pérez-Piñeiro et al. [64] on *Agrobacterium*-mediated transformation summarized the efficient transformation condition into three stages: preculture, inoculation, and co-cultivation. The preculture stage was done to increase the transformation efficiency. Preculture is reported to induce cell division in the explant, thereby enhancing its sensitivity to *Agrobacterium*, which is highly dependent on the duration of preculture [45]. In the wheat cultivar "Bobwhite", the highest T-DNA transfer frequency was achieved when the immature embryos were precultured for ten days based on transient GUS assay [65]. However, Sivanandhan et al. [62] reported that transient GUS expression in *Withania somnifera* (L.) Dunal decreased with a long preculture period, suggesting the importance of determining the optimum preculture duration. To date, no report on pre-culture conditions for oil palm transformation has been available.

The second stage is explant inoculation in an *Agrobacterium* suspension. Yenchon and Te-chato [30] demonstrated that inoculation at an optical density of 0.8 at 600 nm for 6 hours generated the highest number of hygromycin-resistant oil palm embryogenic calli (63.89%) and were able to form somatic embryos after eight weeks of culture. However, no report on transgenic plant regeneration was demonstrated in the paper. Besides cell density, the optimal growth stage of the bacterial culture is also crucial for successful transformation, as infection time also influences transformation efficiencies [21]. The length of contact with *Agrobacterium* suspension cells is a critical step, as the transfer and integration of T-DNA are completed during this period [66]. The highest frequencies of GUS expression and shoot regeneration were observed when leaf segments of Valencia sweet oranges were inoculated with *Agrobacterium* suspension cells for 10 min. However, longer infection duration reduced the transformation frequencies [66]. Likewise, Thiruvengadam et al. [67] stated

that 30 min infection was the optimum for gherkin transformation, and prolonged duration led to overgrowth of *Agrobacterium* and tissue browning. Besides the optimum duration and bacterial density, most researchers have also reported that *Agrobacterium* at the log phase of growth is more efficient than at the lag and stationary phases [49].

The co-cultivation stage in the transformation process has been extensively studied in many crops and plants. In this stage, duration, and temperature of co-cultivation are crucial factors influencing *Agrobacterium*-mediated crop improvement for barley, citrus, and *Withania somnifera* L. [62]. Throughout the co-cultivation period, the addition of acetosyringone as a virulence inducer, sugars as carbon sources, plant growth hormones, and osmoprotectants are crucial to enhance the T-DNA transfer efficiency [64].

3.4 Antioxidants

The inoculation of explants with *Agrobacterium* cells produces reactive oxygen species (ROS) as a response similar to that of a pathogen attack [68]. The ROS causes tissue necrosis, growth inhibition, and altered plant metabolic pathways, resulting in reduced regeneration of transgenic plants [69]. The ROS also induced the expression of pathogenesis-related (PR) proteins, thereby hindering the colonization of *Agrobacterium* and preventing the integration of T-DNA into the plant's genome [70]. The adverse effects of ROS during *Agrobacterium*-mediated transformation and regeneration of transgenic plants can be mitigated by antioxidants. Some of the tested antioxidants are dithiothreitol (DTT) and L-cysteine in maize [71], silver nitrate and sodium thiosulfate in grapevines [70], polyvinylpyrrolidone (PVP) in sorghum [72], tocopherol, ascorbic acid and glutathione in banana [73] and α-lipoic acid in soybean, tomato, *Carrizo citrange* and lentil [23, 74, 75]. Some studies have shown that using surfactants, such as Silweet L-77, can improve T-DNA transfer frequency, as indicated by the number of GUS blue spots in the tested explant [76].

3.5 Regulation of Plant Signaling Molecules

Whilst considerable research has focused on the role of bacterial proteins in plant transformation mediated by *Agrobacterium*, limited investigations have explored the plant-based factors that affect the efficiency of this process [43]. Among these host factors, the phytohormone ethylene, gamma-aminobutyric acid (GABA), and salicylic acid (SA) have been identified as potential targets for improving transformation efficiency [77]. It has been demonstrated that the phytohormone ethylene suppresses T-DNA transfer as well as its incorporation into the host genome, hence impairing *Agrobacterium*-mediated transformation [77, 78]. Similarly, GABA could interfere with the *Agrobacterium* infection process, leading to reduced transformation efficiency [77, 79]. Additionally, SA, which is involved in plant defense responses, may also hinder *Agrobacterium*'s ability to transfer its T-DNA into the plant cell [80]. This was due to the suppression of all virulence (*vir*) genes and *repABC* operon expression in *Agrobacterium,* which are needed to facilitate T-DNA transfer [81]. Moreover, SA is capable of increasing the ROS levels produced during pathogen infection as well as other environmental stresses like temperature, dehydration, and salinity [82]. The regulatory effects of ethylene, GABA, SA, and ROS on *Agrobacterium*-mediated transformation are summarized in Figure 1.

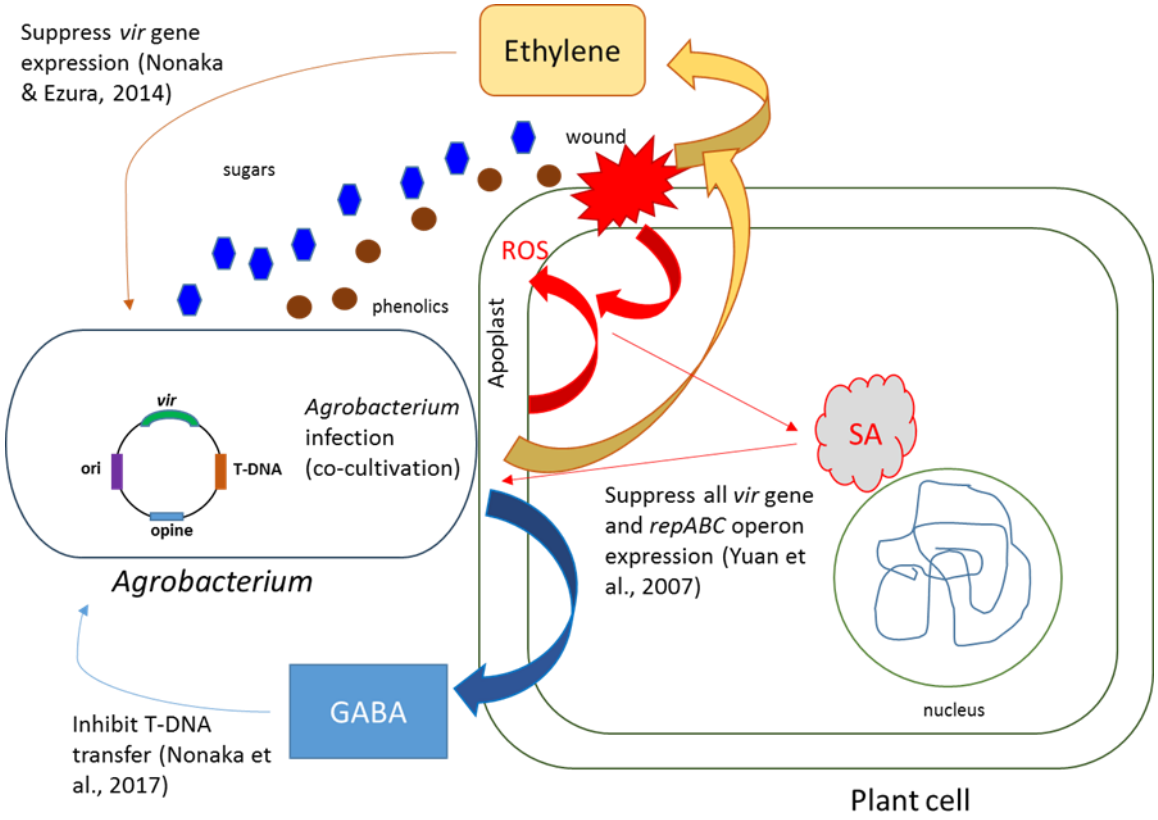

Figure 1 Regulatory effect of reactive oxygen species (ROS), ethylene, GABA, and salicylic acid (SA) on *Agrobacterium*-mediated transformation. Wounding prior to *Agrobacterium* inoculation triggers plant cells to elicit neutral and acidic sugars as well as phenolic compounds to repair the damaged tissues. These molecules attract *Agrobacterium* to infect the plant cells and induce *Agrobacterium* virulence. ROS produced at the infection site increases the infected cells' stress-related plant signaling molecules such as ethylene, GABA, and SA. Ethylene, GABA, and SA suppress *Agrobacterium* virulence (*vir*) gene expression, thus inhibiting the transfer of T-DNA from *Agrobacterium* into the plant cell and consequently reducing transformation efficiency.

Inhibiting the production of these host factors may become a promising scheme to improve the efficacy of *Agrobacterium*-mediated plant transformation. One promising approach involves the utilization of microbial enzymes to modulate key plant metabolites that can influence the transformation process. For example, an *Agrobacterium* strain engineered with 1-Aminocyclopropane-1-carboxylate (ACC) deaminase (AcdS) and GABA transaminase (GabT) enzymes enhanced the efficiency of melon and tomato transformation [79, 83]. The AcdS enzyme will degrade ACC, the ethylene precursor, while the GabT enzyme will degrade GABA [83]. Several studies using transgenic plants expressing the salicylic acid hydroxylase (NahG), an SA metabolizing enzyme, were conducted to investigate the contribution of SA during pathogen attack. The *nahG* gene was transformed into tobacco, *Arabidopsis,* and tomato [84] via *Agrobacterium*-mediated transformation. These NahG transgenic plants were defective in SA due to over-expression of the salicylic acid hydroxylase and were more susceptible to pathogens than the wild-type counterparts. It was observed that the NahG *Arabidopsis* exhibited more intense GUS staining than the

untransformed *Arabidopsis* [84]. Recently, Liu et al. [85] demonstrated that *Arabidopsis* plants carrying the *nahG* gene produced more tumors compared to untransformed *Arabidopsis* plants when infected with the tumorigenic strain *A. tumefaciens* A208, indicating a more profound infection in the mutant. Incorporating these metabolic engineering strategies into *Agrobacterium* strains or the host plants themselves could lead to significant improvements in the success and utility of *Agrobacterium*-mediated transformation for various agricultural and biotechnological applications, such as the development of stress-tolerant plants, production of biopharmaceuticals, and phytoremediation [46].

3.6 Selection Agent

In addition to the transformation procedure, the use of a reliable selection agent is also crucial to producing a stable transgenic plant [46]. The efficacy of geneticin G-418, Basta®, kanamycin, hygromycin, and neomycin has been investigated for oil palm genetic engineering [12]. They concluded that Basta® and hygromycin are suitable for selecting transformants from oil palm embryogenic calli [12]. Later, the Basta® herbicide was used as the selection agent to regenerate Basta-resistant shoots from oil palm embryogenic calli that were transformed using *Agrobacterium* strain LBA4404 [27]. In addition, Parveez et al. [86] and Abdullah et al. [29] reported the suitability of hygromycin in selecting transformed oil palm IEs. The hygromycin gene was effective in selecting transformants in various dicot and monocot plants [87]. Recently, the successful regeneration of *Agrobacterium*-mediated CRISPR/Cas9 edited oil palm using hygromycin selection was reported by Bahariah et al. [18].

3.7 Use of Development Regulator

The ability to regenerate fertile transgenic plants is vital in any plant transformation. Gordon-Kamm et al. [88] stated that the production of transgenic plants involves two stages. The first stage is the incorporation and activation of foreign genes within the host plant (transformation), followed by the development of the modified explant into a fertile transgenic plant through regeneration. Genotype dependency remains a bottleneck in transformation for monocots, particularly in recalcitrant species such as oil palm, maize, rice, sugarcane, cotton, sorghum, wheat, and date palm. One of the most promising tools to overcome this bottleneck is the exploitation of developmental regulator genes such as *Wuschel (WUS)* and Baby *Boom (BBM)*. The *BBM* gene was discovered by Boutilier et al. [89] (2002) in *Brassica napus* during their attempt to identify genes that were upregulated during embryo formation from immature pollen in an in vitro environment. Later, studies in tobacco demonstrated that expression of the *BBM* gene produced sterile transgenic tobacco lines [90]. In *Capsicum annuum*, the *BBM* gene was able to enhance the regeneration of stable transgenic sweet pepper varieties. The transformation efficiencies for the Fiesta and Ferrari sweet pepper varieties were 0.6% and 1.1%, respectively [91].

The utilization of the *BBM* gene in *Agrobacterium*-mediated transformation of monocots was initiated in maize [92]. The findings showed that the *BBM* gene escalated the transformation frequency of two maize inbreds, PHN46 and PH581. The callus transformation frequency of inbred PHN46 increased from 1.7% to 34.9%, while that for inbred PH581 rose from 0.4% to 16.9%. The transformation frequency for both inbreds increased further when the *BBM* gene was combined with the *WUS* gene. Discovery of the *WUS* gene in *Arabidopsis* was reported by Laux et al. [93]. They

concluded that the *WUS* gene is required for leaf and flower formation, as the wus mutant exhibited premature leaf and flower organization. The combination of *BBM* and *WUS* also increased the transformation efficiency of recalcitrant upland switchgrass cultivar Summer to 6% [94]. In palm species such as date, the palm *BBM* (*PdBBM*) improved transformation efficiency to 11% in the Ajwa variety, a variety that had previously failed to develop embryos by promoting embryogenesis and the generation of viable seedlings [95]. However, the use of *BBM* and *WUS* genes resulted in the regeneration of a sterile transgenic plant; therefore, the removal of these genes is essential [92, 96]. The removal of the BBM/WUS combination using the Cre-Lox method was demonstrated to enhance leaf base *Agrobacterium*-mediated transformation of maize and sorghum and subsequently enable genome editing [97].

Besides *BBM* and *WUS* genes, transcription factors, such as Growth Regulating Factors (GRFs), have also been proven to increase the transformation efficiency of monocot species [98]. GRF is involved in various plant development processes, including cell proliferation, overall growth, and maturation, as well as the plant's ability to respond to environmental stress [96]. The activation of the *GRF* gene is modulated by micro RNA miR396. The interaction of GRF with GRF-interacting factors (GIF), known as its cofactor, enhances its function in development regulation [96]. For maize transformation, Kong et al. [98] reported that the maize GRF5 (*ZmGRF5*) gene enhanced callus proliferation, improved transformation efficiency, and resulted in the production of normal, mature maize plants with viable seeds. The study also showed that the inserted gene was successfully transmitted to the T_1 generation. The variety of wheat genotypes that can be transformed and the transformation efficiency were both increased by the co-expression of the *GIF1* and *TaGRF4* genes in wheat [99, 100].

Moreover, the regenerated transgenic *GRF-GIF* wheat was productive and exhibited a normal phenotype. Due to wheat's efficient regeneration without cytokinins, this approach also makes transgenic wheat selectable without the need for a selection agent [99]. The efficacy of the *TaGRF4-GIF* complex in wheat transformation was further improved by altering the miRNA396 target region in the *TaGRF4* gene. Hence, the mutated *TaGRF4* (*mTaGRF4*) enhanced regeneration and genome editing frequency in various wheat [101]. Similarly, the utilization of the *mTaGRF4-GIF1* complex increased the regeneration of shoots and improved the transformation efficiency of the medicinal orchid *Dendrobium catenatum* [102]. Incorporating the *TaGRF4-GIF1* complex into a CRISPR/Cas9 construct for genome editing of wheat enhanced transformation efficiency in numerous wheat varieties and facilitated the production of stably inherited genome-edited wheat [100]. In addition to the abovementioned development regulator genes, recent studies have demonstrated that the *Wuschel-related homeobox 5* (*WOX5*) also significantly improved the development of transgenic plants and subsequently increased the transformation efficiency of numerous monocots, including recalcitrant genotypes [96].

4. *Agrobacterium*-Mediated Trait Improvement via Genome Editing for Sustainability

The oil palm (*E. guineensis* Jacq.) is a crucial crop that provides a significant proportion of the world's edible oil. However, improving the sustainability of oil palm cultivation remains a challenge, as the crop faces issues such as environmental impact, disease susceptibility, and limited genetic diversity. The CRISPR/Cas9 genome editing system has evolved as a cutting-edge technology to address these challenges, allowing for precise modifications to the oil palm genome. CRISPR/Cas9

genome editing has been effectively utilized to enhance nutritional quality, stress tolerance, and disease resistance in a wide range of fruits, vegetables, and ornamentals [103]. This technology holds great potential for enhancing oil palm sustainability through targeted improvements to oil quality, environmental resilience, and resistance to diseases and pests [104, 105].

4.1 Improving Palm Oil Quality

The global demand for vegetable oils has been steadily increasing, with palm oil being one of the most widely consumed and versatile oils. However, consumer perception and acceptance of palm oil have been hindered by concerns over its potential environmental impacts and health implications. One potential solution to address these concerns is to genetically modify palm oil to enhance its nutritional profile, particularly by increasing the oleic acid content. Oleic acid, a monounsaturated fatty acid, is widely recognized for its health benefits. Oleic acid has been shown to reduce the possibility of cardiovascular disease and improve cholesterol levels [106]. Furthermore, oleic acid has been found to possess superior lubricating properties compared to other fatty acids, making it a desirable additive for biobased lubricants [107].

Crude palm oil naturally contains 40% monounsaturated oleic acid, 10% polyunsaturated, and 50% saturated fatty acids. Polyunsaturated fatty acids consist of linolenic acid, while saturated fatty acids comprise 44% palmitic acid and 5% stearic acid [12]. Increasing oleic acid content in palm oil is the primary target of the oil palm biotechnology program [11]. According to the biochemical study, manipulating the expression of β-ketoacyl-ACP synthase II (KASII) and palmitoyl-ACP thioesterase (PAT) is expected to upsurge the level of oleic acid in palm oil [108]. To achieve this target, transformation vectors were constructed to downregulate the PAT and upregulate the KASII genes, and these were delivered into the oil palm ECs [11]. Recently, Bahariah et al. [18] demonstrated the first attempt to target candidate genes for increasing oleic acid in palm oil using CRISPR/Cas9 technology. Regeneration of oil palm plants containing the edited *EgPAT* and oil palm fatty acid desaturase 2 (*EgFAD2*) genes was successful.

Agrobacterium-mediated CRISPR/Cas9 genome editing was successfully employed to upsurge oleic acid levels in soybeans by editing the soybean *FAD2* gene (*GmFAD2*) [109]. In pennycress, the oleic acid level in the seed oil was elevated by CRISPR/Cas9 knockout of the *FAD2* and reduced oleate desaturation 1 (*ROD1*) genes, in combination with a knockout mutation of the fatty acid elongation 1 (*FAE1*) gene [110]. The level of oleic acid in *fad2-fae1* mutants increased to 90%, while that of *rod-fae1* mutants increased to 60% compared with the wild-type pennycress, which contains only 12% of oleic acid [110]. Likewise, improving the levels of stearic, palmitoleic, and ricinoleic acids in palm oil is also important to meet the diverse requirements of the oil palm sector [11, 12].

4.2 Improving Oil Palm Height

The ability of CRISPR/Cas9 to generate dwarf and semidwarf plants such as rice [111, 112], rapeseed [113], maize [114], and cucumber [115] by manipulating height and gibberellic acid (GA) related genes has been reported. In the context of oil palm, the development of dwarf oil palms through targeted gene editing can provide several benefits for sustainable cultivation. Shorter palm heights can facilitate a more straightforward harvesting process and reduce the risk of mechanical damage during operations [116]. Additionally, the reduced stature can lead to more efficient use of

land and resources, ultimately enhancing the overall economic performance and viability of oil palm cultivation areas [104, 116].

Genes and transcription factors associated with oil palm height were successfully identified [117, 118]. Among the genes are brassinosteroid insensitive 1-associated receptor kinase 1 precursor (*BRI1*), gibberellin receptor (*GID1*), late elongated hypocotyl protein (*LHY*), E3 ubiquitin-protein ligase (*E3Ub*) and 24-methyltransferase 1 (*SMT1*). These genes regulate different steps in gibberellins (GA) and brassinosteroids (BR) biosynthesis, as well as signaling pathways for plant development [117, 119]. Functional studies for three oil palm GA-related genes, *EgGA20ox*, *EgGA2ox,* and *EgGAI,* have been done using *Arabidopsis thaliana* as the model plant. The results revealed that downregulation of the *EgGA20ox* gene decreased the height of *A. thaliana* as well as the length of the root, leaves, and silique [120].

In the *indica* rice cultivar, modification of *OsGA20ox2* via CRISPR/Cas9 resulted in semidwarf rice with decreased stature, lower GA levels, and a smaller flag leaf dimension. Additionally, the yield per plant increased to up to 6% [111]. Likewise, gene editing of the *semidwarf 1* (*SD1*) gene, which encodes the gibberellin 20-oxidase 2 (GA20ox2) enzyme, using CRISPR/Cas9 technology reduced the height of Xiangdaowan rice [112]. Another gene targeted to produce a dwarf phenotype was *CsERECTA*, which encodes an LRR receptor-like serine/threonine-protein kinase that controls the extension of the stem in cucumber (*Cucumis sativus*) [115]. Knockout mutants of this gene produced dwarf cucumber plants with reduced expression of GA-related genes, resulting in decreased GA levels. Other than GA-related genes, BR-related genes were also targeted for genome editing using CRISPR/Cas9. For instance, a single mutation in the soybean (Glycine max) *GmDWF1a* and *GmDWF1b* genes, which are essential for BR synthesis, produced moderate dwarfism. In contrast, a double mutant of both genes produced a higher degree of dwarfism. Moreover, the single mutant of *GmDWF1a* yielded more soybean pods than its wild-type counterpart in the field test [121]. This information leads to a more comprehensive understanding of the genes controlling plant height and may be used in CRISPR/Cas9-mediated production of dwarf palms. For example, Yeap et al. [17] targeted brassinosteroid-insensitive 1 (*EgBRI1*) genes in an attempt to generate dwarf palm. However, no data on the regeneration of dwarf palm was reported.

4.3 Improving Commercial Oil Palm Fruit Color

Breeding for the *virescens* oil palm trait has gained interest in oil palm breeders due to its prominent color changes as compared to the common *nigrescens* palm [122]. The color of the fruit serves as a crucial cue for bunch ripeness to obtain maximum oil yield. The *nigrescens* palm fruits have deep-violet apex, and the color changes to red with a taint of violet when they ripen. Meanwhile, the unripe *virescens* palm fruits are fully green, and the color changes to bright orange with some residual green at the apical region [123]. These distinct color changes help a harvester to easily spot the ripe bunches, especially for tall palms [124]. The *virescens* palms typically exhibit delayed fruit ripening, which can extend the harvest window and reduce waste due to overripening [116]. This trait can also be introduced into non-abscising oil palms to facilitate the harvesting of ripe fruit bunches, as the loose fruit standard is not applicable to bunches that do not shed fruits [124].

The oil palm *virescens (EgVIR)* gene responsible for controlling oil palm fruit color was identified by Singh et al. [123]. This gene encodes the R2R3-MYB transcription factor, which controls oil palm

fruit color by regulating the expression of genes associated with anthocyanin biosynthesis. The *EgVIR* gene shares homology with *Lilium* (lily) *LhMYB12.* The *EgVIR* gene is also similar to the Production of Anthocyanin Pigment 1 (*PAP1*) and *AtMYB113* of *Arabidopsis*. Further analysis of the *EgVIR* gene has identified five independent mutations in exon 3 that are responsible for the *virescens* fruit color [123]. These mutations resulted in the production of truncated proteins of the R2R3-MYB transcription factor. Notably, these mutations were not observed in the *nigrescens* palm. Hence, this information could assist in the development of CRISPR/Cas9 vectors to produce *virescens* oil palm. In 2019, Aprilyanto and colleagues successfully constructed a CRISPR/Cas9 vector carrying a gRNA targeting the *EgVIR* gene; however, no further results were reported [125].

In other fruits, alteration of the color-related gene sequences produced fruits with colors different from the usual wild type. For example, in tomato (*Solanum lycopersicum*) cv Ailsa Craig, the use of *Agrobacterium*-mediated CRISPR/Cas9 to eliminate genes involved in the carotenoid biosynthesis pathway altered levels of different carotenoids depending on which gene was targeted [126]. For instance, the knockout of the stay-green 1 (*SGR1*) gene increased the lycopene content five-fold compared to the wild type and enhanced the color of the tomato fruit [126]. Besides carotenoid biosynthesis, genes associated with flavonoid biosynthesis were also targeted to modify fruit color. In particular, knockout mutation of the tomato R2R3-MYB transcription factor (*MYB12*) gene inhibited the accumulation of naringenin chalcone (NarCh), one of the flavonoids that contributed to the red color of ripe tomato fruit. This mutation resulted in a pink tomato fruit [127]. Recently, genome editing via multiplex CRISPR/Cas9 of the tomato phytoene synthase 1 (*PSY1*), *SGR1,* and *MYB1* genes produced light green tomato fruit due to decreased levels of lycopene, β-carotene and NarCh and increased chlorophyll content. However, despite the changes in fruit color, the agronomic traits such as plant growth, flowering time, ripening time, and tomato yield were similar to those of the wild type [128].

4.4 Improving Oil Palm Resilience to Environmental Stress

Globally, the agricultural sector faces significant challenges in ensuring food security due to the detrimental effects of changing weather patterns. The oil palm industry is not immune to these challenges, as it is susceptible to the effects of drought and other environmental stresses [116]. The unfavorable conditions for oil palm growth exposed the palm to abiotic stress, affecting its productivity [129]. The oil palm industry's capacity to develop climate-resilient varieties has been hindered by the narrow genetic base of the commonly grown Deli *dura* and AVROS *pisifera* populations [130]. Genes that exhibit varying levels of expression under different abiotic stress conditions have been isolated and characterized, as reported by Wei et al. [129]. Briefly, the oil palm MYB (*EgMYB*), some members of the *EgARF* family, and *bZIP* genes were upregulated during cold, drought, and salinity stresses [130]. The *EgLEA4* gene also has the ability to increase drought resistance [131].

CRISPR/Cas9 technology has been utilized to investigate genes linked to abiotic stress tolerance in various crops, including maize, tomato, rice, potato, and cotton [132, 133]. Knockout mutants may have increased resistance or tolerance to the abiotic stress conditions depending on which gene was edited. As an example, enhancing rice's cold stress tolerance was achieved by utilizing *Agrobacterium*-mediated CRISPR/Cas9 to eliminate the cold-responsive R2R3-type MYB transcription factor, which is encoded by the *OsMYB30* gene [134]. In wheat, CRISPR/Cas9 knockout

of the *TaSal1* gene improved wheat tolerance to drought [135]. Furthermore, CRISPR/Cas9 knockout of the maize abscisic acid-, stress-, and ripening-induced (ASR) gene (*ZmASR1*) enhanced maize tolerance to drought [136].

4.5 Improving Oil Palm Resistance to Disease

The oil palm sector faces significant challenges in addressing diseases that pose a substantial threat to productivity and sustainability. One such disease is the Basal Stem Rot (BSR), which is triggered by *Ganoderma boninense*, a fungal pathogen that can cause yield losses of 50%-80% [116]. In Malaysia, the BSR disease affects young palms and seedlings, as well as mature palms [137]. In addition to BSR, oil palms are also susceptible to infection by *Phytophthora palmivora*, an oomycete that triggered the bud rot disease [138]. This pathogen can attack various monocots and dicots, including vegetables and trees, significantly reducing plant production [139]. In Southeast Asia, among the plants that are affected by *P. palmivora* are coffee (*Coffee arabica*), oil palm (*E. guineensis*), black pepper (*Piper nigrum* L.), rubber (*Hevea brassiliensis*) and durian (*Durio zibethinus*) [140].

To enhance disease resistance in plants, researchers can target the plant susceptibility (*S*) gene using CRISPR/Cas9 genome editing, thereby affecting the plant-pathogen interaction and preventing infection [141]. Other possible targets for disease resistance are the resistance (*R*) gene, hormonal pathway, and pathogen virulence factor [142]. This technology has successfully produced a variety of plants with increased resistance to viruses, bacteria, fungi, and oomycetes, as listed in Zaidi et al. [143]. For example, the inactivation of the *OsERF922* gene, which encodes an ethylene-responsive factor (ERF) transcription factor, in the japonica rice cultivar Kuiku 131 led to improved resistance against rice blast disease caused by the fungal *Magnaporthe oryzae* [144]. The mutation was stable and successfully inherited in the T_2 generation. The important agronomic traits, such as plant height, length of the flag leaf, quantity of productive panicles, and number of grains in each panicle, were similar to those of the wild type. A recent investigation demonstrated that the elimination of the MAP kinase phosphatase 1 (*TaMKP1*) gene in wheat (*T. aestivum*) through CRISPR/Cas9 technology led to improved resistance against two fungal pathogens. The study showed increased protection against *Blumeria graminis* f. sp. *tritici* (*Bgt*), which caused powdery mildew, and *Puccinia striiformis* f. sp. *tritici* (*Pst*) that caused the rust disease [145]. Unexpectedly, the modified wheat plants were taller and produced more grains than their unaltered counterparts.

For the diseases manifested by the infection of oomycetes (*Phytophthora*), several genes related to plant immunity were edited to enhance resistance against the pathogen. One of the genes is the cacao tree (*Theobroma cacao*) non-expressor pathogenesis related 3 (*TcNPR3*). The *TcNPR3* gene is a defense response repressor gene, and the knockout of this gene increased endurance to *P. tropicalis* [146]. In another study, Raf-like protein was targeted for CRISPR/Cas9 genome editing to enhance resistance against *P. parasitica* for *A. thaliana*. The CRISPR/Cas9 genome editing of the *A. thaliana Raf36* gene resulted in a frameshift mutation, which reduced gene expression and produced a truncated Raf36 protein. When the detached leaf of the *raf36* mutant was inoculated with *P. parasitica,* the lesion was smaller than the control, indicating enhanced resistance to the pathogen [147]. Recently, a group of scientists from China successfully increased the resistance of soybean (*Glycine max* L.) plant to the oomycete *P. sojae* via *Agrobacterium*-mediated CRISPR/Cas9 knockout of the soybean acetyltransferase gene (*GmTAP1*). Notably, the edited soybean plant

exhibited similar tallness, pod number per plant, and yield per plant as the wild type in the field test, confirming that knockout of the *GmTAP1* gene did not affect the agronomic traits [148].

4.6 Improving Oil Palm Resistance to Pest

The potential of CRISPR/Cas9 technology for managing insect pests was also explored and critically reviewed by Komal et al. [149]. Interestingly, CRISPR-mediated insect pest management can be achieved by modifying either plant or insect genomes, as suggested by Tyagi et al. [150]. However, this review will focus solely on CRISPR/Cas9 modification of the plant genome to provide insights into potential genes for enhancing oil palm resistance to pests. Creating insect-resistant crops through genome editing techniques will reduce farmers' reliance on pesticides. This shift is beneficial, as pesticide use can lead to various problems, including the emergence of pesticide-resistant insects, contamination of soil and water resources, and heightened expenses for managing environmental health concerns [151].

As a natural defense mechanism, plants synthesize metabolic substances in response to insect pest attacks. Among them are serotonin and salicylic acid [152]. In plants, both serotonin and salicylic acid are synthesized from chorismate, a metabolite from the shikimate pathway [153]. Isochorismate can be produced from chorismate, which can then be transformed into salicylic acid. Chorismate is also converted to tryptophan, which is subsequently converted to tryptamine for the synthesis of serotonin [153]. In a 2018 study, Lu et al. [154] demonstrated the creation of rice with enhanced resistance to insects by employing CRISPR/Cas9 techniques to inhibit the production of serotonin. They inactivated the *CYP71A1* gene, which encodes the tryptamine-5-hydroxylase, an enzyme responsible for converting tryptamine into serotonin. Consequently, the lack of serotonin in rice enhanced its ability to resist stem borers and plant hoppers [154]. Subsequently, Wang et al. [155] conducted additional research on these mutants, which substantiated that rice plants with a reduced serotonin level confer increased resistance against striped stem borer.

Flavonoids are another type of plant metabolite that can protect plants from insect pests and pathogen attacks [156]. Numerous investigations have shown that CRISPR/Cas9 knockout of certain flavonoid biosynthesis genes awarded plants with insect resistance properties. For example, in the soybean cultivar Tianlong No.1, modification of the *UDP-glycosyltransferase* gene (*GmUDP*) via *Agrobacterium*-mediated CRISPR/Cas9 conferred enhanced resistance to the leaf-eating pests *Helicoverpa armigera* and *Spodoptera litura* [151]. The UDP-glycosyltransferase (UDP) is a cytosolic enzyme involved in generating flavonoid derivatives in the last stage of flavonoid biosynthesis. Mutation in the *GmUDP* gene altered the flavonoid content in the soybean leaf, and long-term feeding of *H. armigera* with the mutant soybean leaves reduced the larvae's immunity [151]. In addition, the *Agrobacterium*-mediated CRISPR/Cas9 knockout of the *Nipponbare* rice *naringenin O-glucosyltransferase* gene changed the flavonoid content in the rice. This modification leads to a reduced loss by two rice-consuming pests, brown plant hopper (*Nilaparvata lugens*) and rice grasshopper (*Oxya hyla intricate*) [102].

5. Conclusion and Prospects

The ongoing enhancement of oil palm planting materials has been facilitated by insights from oil palm physiology and molecular biology studies, which focus on genes that regulate growth, oil yield, and stress responses. This improvement process incorporates conventional breeding, genetic

engineering, and RNAi-mediated gene silencing coupled with tissue culture for regeneration and propagation [13, 54]. The complete mapping of the oil palm genome [16] has paved the way for the improvement of oil palm planting materials via CRISPR/Cas9 technology. Additionally, CRISPR/Cas9 genome editing studies on other crops offer valuable lessons for enhancing oil palm agronomic traits. Present efforts to apply CRISPR/Cas9 technology to improve oil palm planting materials employ various DNA delivery methods, including biolistic, *Agrobacterium*-mediated transformation [18, 104, 105], and PEG-mediated protoplast transformation [20]. While CRISPR/Cas9 technology has demonstrated successful genome editing of oil palms [17, 18, 20], enhancing *Agrobacterium*-mediated transformation efficiency would significantly accelerate the production of edited oil palms. Furthermore, developing transgene-free genome editing via *Agrobacterium*-mediated transformation could improve consumer acceptance of genome-edited oil palms. In essence, the strategic implementation of CRISPR/Cas9-mediated genome editing through *Agrobacterium*-based transformation presents significant opportunities for developing more sustainable oil palm planting materials, which could potentially contribute to the sustainability and environmental responsibility of the oil palm sector.

Acknowledgments

The authors thank the MPOB management for permission to publish this review. The authors would also like to acknowledge all the Transgenic Technology Group (TTG) members of MPOB for their advice and kind assistance.

Author Contributions

Hanin AN conceptualized the idea, conducted literature surveys, and drafted the manuscript. Masani MYA conceived the idea, edited and finalized the write-up of the manuscript, and submitted it. Rasid OA, Janna OA, and Parveez GKA reviewed and edited the manuscript. All authors read and approved the final manuscript.

Funding

No funding was applied for this review.

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the literature reported in this review paper.

References

1. Tiseo I. Global vegetable oil yields per hectare worldwide 2020, by type [Internet]. New York, NY: Statista Inc.; 2025. Available from: https://www.statista.com/statistics/1263201/vegetable-oil-yields-per-hectare-worldwide-by-type-globally/#statisticContainer.
2. Parveez GK, Leow SS, Kamil NN, Madihah AZ, Ithnin MA, Ng MH, et al. Oil palm economic performance in Malaysia and R&D progress in 2023. J Oil Palm Res. 2024; 36: 171-186.

3. Shahbandeh M. Palm oil top global producers 2024/25 [Internet]. New York, NY: Statista Inc.; 2025. Available from: https://www.statista.com/statistics/856231/palm-oil-top-global-producers/.
4. Widmar D. Two charts: Global vegetable oil trends [Internet]. Lafayette, IN: Agricultural Economic Insights; 2022. Available from: https://aei.ag/overview/article/vegetable-oil-trends-production-oilseeds.
5. OECD-FAO. Oilseeds and oilseed products. In: OECD-FAO agricultural outlook 2023-2032. Paris, France: OECD Publishing; 2023. pp. 158-169.
6. Wong A, Choy R. Crude palm oil outlook: MARC Ratings forecasts slightly positive trend [Internet]. Kuala Lumpur, Malaysia: MARC; 2023. Available from: https://www.marc.com.my/views/crude-palm-oil-outlook-marc-ratings-forecasts-slightly-positive-trend/.
7. Sambanthamurthi R, Singh R, Kadir AP, Abdullah MO, Kushairi A. Opportunities for the oil palm via breeding and biotechnology. In: Breeding plantation tree crops: Tropical species. New York, NY: Springer New York; 2009. pp. 377-421.
8. Kushairi A, Singh R, Ong-Abdullah M. The oil palm industry in Malaysia: Thriving with transformative technologies. J Oil Palm Res. 2017; 29: 431-439.
9. Zulkifli Y, Norziha A, Naqiuddin MH, Fadila AM, Nor Azwani AB, Suzana M, et al. Designing the oil palm of the future. J Oil Palm Res. 2017; 29: 440-455.
10. Parveez GK, Bahariah B, Ayub NH, Masani MY, Rasid OA, Tarmizi AH, et al. Production of polyhydroxybutyrate in oil palm (*Elaeis guineensis* Jacq.) mediated by microprojectile bombardment of PHB biosynthesis genes into embryogenic calli. Front Plant Sci. 2015; 6: 598.
11. Parveez GK, Rasid OA, Masani MY, Sambanthamurthi R. Biotechnology of oil palm: Strategies towards manipulation of lipid content and composition. Plant Cell Rep. 2015; 34: 533-543.
12. Masani MY, Izawati AM, Rasid OA, Parveez GK. Biotechnology of oil palm: Current status of oil palm genetic transformation. Biocatal Agric Biotechnol. 2018; 15: 335-347.
13. Rasid OA, Masura SS, Hanin AN, Masli DI, Bohari B, Masani MY, et al. Oil palm transgenic research: Challenges, update, and future outlook. In: The oil palm genome. Cham: Springer; 2020. pp. 69-81.
14. Tan LW, Rahman ZA, Goh HH, Hwang DJ, Ismail I, Zainal Z. Production of transgenic rice (*indica cv*. MR219) overexpressing *Abp57* gene through *Agrobacterium*-mediated transformation. Sains Malays. 2017; 46: 703-711.
15. Ahmed RI, Ding A, Xie M, Kong Y. Progress in optimization of *Agrobacterium*-mediated transformation in sorghum (*Sorghum bicolor*). Int J Mol Sci. 2018; 19: 2983.
16. Singh R, Ong-Abdullah M, Low ET, Manaf MA, Rosli R, Nookiah R, et al. Oil palm genome sequence reveals divergence of interfertile species in old and new worlds. Nature. 2013; 500: 335-339.
17. Yeap WC, Khan NC, Jamalludin N, Muad MR, Appleton DR, Kulaveerasingam H. An efficient clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein 9 mutagenesis system for oil palm (*Elaeis guineensis*). Front Plant Sci. 2021; 12: 773656.
18. Bahariah B, Masani MY, Fizree MP, Abd Rasid O, Parveez GK. Multiplex CRISPR/Cas9 gene-editing platform in oil palm targeting mutations in *EgFAD2* and *EgPAT* genes. J Genet Eng Biotechnol. 2023; 21: 3.

19. Jamaludin N, Bahariah B, Shaharuddin NA, Ho CL, Rasid OA, Parveez GK, et al. Designing gRNAs targeting oil palm phytoene desaturase (*EgPDS*) gene and construction of vectors for oil palm CRISPR/Cas9 study. J Oil Palm Res. 2022; 35: 133-146.
20. Norfaezah J, Masani MY, Fizree MP, Bahariah B, Shaharuddin NA, Ho CL, et al. DNA-free CRISPR/Cas9 genome editing system for oil palm protoplasts using multiple ribonucleoproteins (RNPs) complexes. Ind Crops Prod. 2024; 208: 117795.
21. Gelvin SB. Traversing the cell: *Agrobacterium* T-DNA's journey to the host genome. Front Plant Sci. 2012; 3: 52.
22. Nester EW. *Agrobacterium*: Nature's genetic engineer. Front Plant Sci. 2015; 5: 730.
23. Azizi-Dargahlou S, Pouresmaeil M. *Agrobacterium tumefaciens*-mediated plant transformation: A review. Mol Biotechnol. 2024; 66: 1563-1580.
24. Hiei Y, Ishida Y, Komari T. Progress of cereal transformation technology mediated by *Agrobacterium tumefaciens*. Front Plant Sci. 2014; 5: 628.
25. Yamada T, Takagi K, Ishimoto M. Recent advances in soybean transformation and their application to molecular breeding and genomic analysis. Breed Sci. 2012; 61: 480-494.
26. Lobato-Gómez M, Hewitt S, Capell T, Christou P, Dhingra A, Girón-Calva PS. Transgenic and genome-edited fruits: Background, constraints, benefits, and commercial opportunities. Hortic Res. 2021; 8: 166.
27. Masli DI, Kadir AP, Yunus AM. Transformation of oil palm using *Agrobacterium tumefaciens*. J Oil Palm Res. 2009; 21: 643-652.
28. Song GQ, Prieto H, Orbovic V. *Agrobacterium*-mediated transformation of tree fruit crops: Methods, progress, and challenges. Front Plant Sci. 2019; 10: 226.
29. Abdullah R, Zainal A, Yew Heng W, Chui Li L, Chee Beng Y, Mei Phing L, et al. Immature embryo: A useful tool for oil palm (*Elaeis guineensis* Jacq.) genetic transformation studies. Electron J Biotechnol. 2005; 8: 24-34.
30. Yenchon S, Te-chato S. Effect of bacteria density, inoculation and co-cultivation period on *Agrobacterium*-mediated transformation of oil palm embryogenic callus. J Agric Technol. 2012; 8: 1485-1496.
31. Izawati AM, Masani MY, Ismanizan I, Parveez GK. Evaluation on the effectiveness of 2-deoxyglucose-6-phosphate phosphatase (*DOG^{R}1*) gene as a selectable marker for oil palm (*Elaeis guineensis* Jacq.) embryogenic calli transformation mediated by *Agrobacterium tumefaciens*. Front Plant Sci. 2015; 6: 727.
32. Hanin AN, Masani MY, Rasid OA, Parveez GK. Vector construction and transient evaluation in oil palm calli via *Agrobacterium*-mediated transformation. J Oil Palm Res. 2024; 36: 210-223.
33. Liu H, Xie X, Sun S, Zhu W, Ji J, Wang G. Optimization of '*Agrobacterium*'-mediated transformation of sunflower (*Helianthus annuus* L.) immature embryos. Aust J Crop Sci. 2011; 5: 1616-1621.
34. Bates R, Craze M, Wallington EJ. *Agrobacterium*-mediated transformation of oilseed rape (*Brassica napus*). Curr Protoc Plant Biol. 2017; 2: 287-298.
35. Promchan T, Te-Chato S. Strians of *Agrobacterium* affecting gene transformation through embryogenic cell suspension of hybrid tenera oil palm. Int J Agric Technol. 2013; 9: 669-679.
36. Fuad FA, Ismail I, Sidik NM, Zain CR, Abdullah R. Super binary vector system enhanced transformation frequency and expression level of polyhydroxyvalerate gene in oil palm immature embryo. Asian J Plant Sci. 2008; 7: 526-535.

37. Yenchon S, Te-chato S. Sonication-assisted *Agrobacterium*-mediated gene transformation of oil palm secondary somatic embryo. Agric Nat Resour. 2015; 49: 319-326.
38. Le V. Co-transformation of oil palm using *Agrobacterium*-mediated transformation. Nat Proc. 2010. doi: 10.1038/npre.2010.5441.1.
39. Ismail I, Iskandar NF, Chee GM, Abdullah R. Genetic transformation and molecular analysis of polyhydroxybutyrate biosynthetic gene expression in oil palm ('*Elaeis guineensis*' Jacq. var '*Tenera*') Tissues. Plant Omics. 2010; 3: 18-27.
40. Ariffin N, Abdullah R, Muad MR, Lourdes J, Emran NA, Ismail MR, et al. Constructions of expression vectors of polyhydroxybutyrate-co-hydroxyvalerate (PHBV) and transient expression of transgenes in immature oil palm embryos. Plasmid. 2011; 66: 136-143.
41. Li SY, Zhang Q, Jin YH, Zou JX, Zheng YS, Li DD. A MADS-box gene, *EgMADS21*, negatively regulates *EgDGAT2* expression and decreases polyunsaturated fatty acid accumulation in oil palm (*Elaeis guineensis* Jacq.). Plant Cell Rep. 2020; 39: 1505-1516.
42. Minarsih H, Permatasari GW, Setiawati Y, Mardhika LD, Saptari RT, Riyadi I, et al. Transformation of CRISPR/Cas9 expression construct to enhance saturated fatty acid synthesis in oil palm for biofuel production. IOP Conf Ser Earth Environ Sci. 2023; 1255: 012058.
43. Tzfira T, Citovsky V. *Agrobacterium*-mediated genetic transformation of plants: Biology and biotechnology. Curr Opin Biotechnol. 2006; 17: 147-154.
44. Karami O. Factors affecting *Agrobacterium*-mediated transformation of plants. Transgenic Plant J. 2008; 2: 127-137.
45. Kumar N, Vijayanand KG, Reddy MP, Singh AS, Naraynan S. *Agrobacterium tumefaciens*-mediated genetic transformation: Mechanism and factors. J For Environ Scie. 2009; 25: 195-204.
46. Ziemienowicz A. *Agrobacterium*-mediated plant transformation: Factors, applications and recent advances. Biocatal Agric Biotechnol. 2014; 3: 95-102.
47. Li S, Cong Y, Liu Y, Wang T, Shuai Q, Chen N, et al. Optimization of *Agrobacterium*-mediated transformation in soybean. Front Plant Sci. 2017; 8: 246.
48. Singh P, Khan S, Kumar S, ur Rahman L. Establishment of an efficient *Agrobacterium*-mediated genetic transformation system in *Pelargonium graveolens*: An important aromatic plant. Plant Cell Tissue Organ Cult. 2017; 129: 35-44.
49. Saha P, Blumwald E. Spike-dip transformation of *Setaria viridis*. Plant J. 2016; 86: 89-101.
50. Shrawat AK, Lörz H. *Agrobacterium*-mediated transformation of cereals: A promising approach crossing barriers. Plant Biotechnol J. 2006; 4: 575-603.
51. Opabode JT. *Agrobacterium*-mediated transformation of plants: Emerging factors that influence efficiency. Biotechnol Mol Biol Rev. 2006; 1: 12-20.
52. Huang X, Wei Z. Successful *Agrobacterium*-mediated genetic transformation of maize elite inbred lines. Plant Cell Tissue Organ Cult. 2005; 83: 187-200.
53. Boonyaves K, Romyanon K, Kirdmanee C, Supaibulwatana K. *Agrobacterium tumefaciens*-mediated genetic transformation in oil palm (*Elaeis guineensis* Jacq.). Proceedings of the Agricultural Biotechnology International Conference: Agricultural biotechnology for better living and a clean environment; 2009 September 22-25; Queen Sirikit National Convention Center, Bangkok, Thailand. Nakhon Pathom, Thailand: College of Agriculture.

54. Fu Q, Li C, Tang M, Tao YB, Pan BZ, Zhang L, et al. An efficient protocol for *Agrobacterium*-mediated transformation of the biofuel plant *Jatropha curcas* by optimizing kanamycin concentration and duration of delayed selection. Plant Biotechnol Rep. 2015; 9: 405-416.
55. Chetty VJ, Ceballos N, Garcia D, Narváez-Vásquez J, Lopez W, Orozco-Cárdenas ML. Evaluation of four *Agrobacterium tumefaciens* strains for the genetic transformation of tomato (*Solanum lycopersicum* L.) cultivar Micro-Tom. Plant Cell Rep. 2013; 32: 239-247.
56. Ribas AF, Dechamp E, Champion A, Bertrand B, Combes MC, Verdeil JL, et al. *Agrobacterium*-mediated genetic transformation of *Coffea arabica* (L.) is greatly enhanced by using established embryogenic callus cultures. BMC Plant Biol. 2011; 11: 92.
57. Huang X, Huang XL, Xiao W, Zhao JT, Dai XM, Chen YF, et al. Highly efficient *Agrobacterium*-mediated transformation of embryogenic cell suspensions of *Musa acuminata* cv. Mas (AA) via a liquid co-cultivation system. Plant Cell Rep. 2007; 26: 1755-1762.
58. Parveez GKA, Singh R, Ong-Abdullah M, Ramli US, Low ETL, Rasid OA, et al. Impact of biotechnology on the sustainable development of the oil palm industry-from research to application. In: PIPOC 2017 International Palm Oil Congress - Agriculture, Biotechnology & Sustainability Conference Vol. I. Malaysia: Malaysian Palm Oil Board; 2017. pp. 77-86.
59. Rasid OA, Masani MYA, Masura SS, Bahariah B, Hanin AN, Lim FH, et al. Genetic engineering of oil palm. In: Advances in oil palm research. 2nd ed. Malaysia: MPOB Press; 2023. pp. 91-128.
60. Beranová M, Rakouský S, Vávrová Z, Skalický T. Sonication assisted *Agrobacterium*-mediated transformation enhances the transformation efficiency in flax (*Linum usitatissimum* L.). Plant Cell Tissue Organ Cult. 2008; 94: 253-259.
61. Subramanyam K, Subramanyam K, Sailaja KV, Srinivasulu M, Lakshmidevi K. Highly efficient *Agrobacterium*-mediated transformation of banana cv. Rasthali (AAB) via sonication and vacuum infiltration. Plant Cell Rep. 2011; 30: 425-436.
62. Sivanandhan G, Kapil Dev G, Theboral J, Selvaraj N, Ganapathi A, Manickavasagam M. Sonication, vacuum infiltration and thiol compounds enhance the *Agrobacterium*-mediated transformation frequency of *Withania somnifera* (L.) Dunal. PLoS One. 2015; 10: e0124693.
63. Kayim M, Koc NK. Improved transformation efficiency in citrus by plasmolysis treatment. J Plant Biochem Biotechnol. 2005; 14: 15-20.
64. Pérez-Piñeiro P, Gago J, Landín M, Gallego PP. *Agrobacterium*-mediated transformation of wheat: General overview and new approaches to model and identify the key factors involved. Transgenic plants-advances and limitations. Rijeka, Croatia: Intech Open Access Publisher; 2012.
65. Uzé M, Potrykus I, Sautter C. Factors influencing T-DNA transfer from *Agrobacterium* to precultured immature wheat embryos (*Triticum aestivum* L.). Cereal Res Commun. 2000; 28: 17-23.
66. Khan EU, Fu XZ, Liu JH. *Agrobacterium*-mediated genetic transformation and regeneration of transgenic plants using leaf segments as explants in Valencia sweet orange. Plant Cell Tissue Organ Cult. 2012; 109: 383-390.
67. Thiruvengadam M, Jeyakumar J, Kamaraj M, Chung IM, Kim JJ. Optimization of *Agrobacterium*-mediated genetic transformation in gherkin ('*Cucumis anguria*' L.). Plant Omics. 2013; 6: 231-239.
68. Kuta DD, Tripathi L. *Agrobacterium*-induced hypersensitive necrotic reaction in plant cells: A resistance response against *Agrobacterium*-mediated DNA transfer. Afr J Biotechnol. 2005; 4: 752-757.

69. Dan Y. Biological functions of antioxidants in plant transformation. In Vitro Cell Dev Biol Plant. 2008; 44: 149-161.
70. Nookaraju A, Agrawal DC. Enhanced tolerance of transgenic grapevines expressing chitinase and *β-1,3-glucanase* genes to downy mildew. Plant Cell Tissue Organ Cult. 2012; 111: 15-28.
71. Du H, Wu H, Yan J, Li J. Effects of basal media, salt concentrations, antioxidant supplements and co-effects on the *Agrobacterium*-mediated transformation efficiency in maize. Afr J Biotechnol. 2010; 9: 1135-1143.
72. Sato-Izawa K, Tokue K, Ezura H. Development of a stable *Agrobacterium*-mediated transformation protocol for Sorghum bicolor Tx430. Plant Biotechnol. 2018; 35: 181-185.
73. Namukwaya B, Magambo B, Arinaitwe G, Oweitu C, Erima R, Tarengera D, et al. *Antioxidants* enhance banana embryogenic cell competence to *Agrobacterium* mediated transformation. Afr J Biotechnol. 2019; 18: 735-743.
74. Dan Y, Zhang S, Zhong H, Yi H, Sainz MB. Novel compounds that enhance *Agrobacterium*-mediated plant transformation by mitigating oxidative stress. Plant Cell Rep. 2015; 34: 291-309.
75. Polowick PL, Yan W. A protocol for *Agrobacterium*-mediated genetic transformation of *Lens culinaris* Medik (lentil). Plant Cell Tissue Organ Cult. 2023; 152: 605-618.
76. Wu H, Sparks C, Amoah B, Jones HD. Factors influencing successful *Agrobacterium*-mediated genetic transformation of wheat. Plant Cell Rep. 2003; 21: 659-668.
77. Karami O, Esna-Ashari M, Karimi Kurdistani G, Aghavaisi B. *Agrobacterium*-mediated genetic transformation of plants: The role of host. Biol Plant. 2009; 53: 201-212.
78. Nonaka S, Ezura H. Plant-*Agrobacterium* interaction mediated by ethylene and super-*Agrobacterium* conferring efficient gene transfer. Front Plant Sci. 2014; 5: 681.
79. Nonaka S, Someya T, Zhou S, Takayama M, Nakamura K, Ezura H. An *Agrobacterium tumefaciens* strain with gamma-aminobutyric acid transaminase activity shows an enhanced genetic transformation ability in plants. Sci Rep. 2017; 7: 42649.
80. Subramoni S, Nathoo N, Klimov E, Yuan ZC. *Agrobacterium tumefaciens* responses to plant-derived signaling molecules. Front Plant Sci. 2014; 5: 322.
81. Yuan ZC, Edlind MP, Liu P, Saenkham P, Banta LM, Wise AA, et al. The plant signal salicylic acid shuts down expression of the *vir* regulon and activates quormone-quenching genes in *Agrobacterium*. Proc Natl Acad Sci. 2007; 104: 11790-11795.
82. Herrera-Vásquez A, Salinas P, Holuigue L. Salicylic acid and reactive oxygen species interplay in the transcriptional control of defense genes expression. Front Plant Sci. 2015; 6: 171.
83. Nonaka S, Someya T, Kadota Y, Nakamura K, Ezura H. Super-*Agrobacterium* ver. 4: Improving the transformation frequencies and genetic engineering possibilities for crop plants. Front Plant Sci. 2019; 10: 1204.
84. Rosas-Díaz T, Cana-Quijada P, Amorim-Silva V, Botella MA, Lozano-Durán R, Bejarano ER. *Arabidopsis* NahG plants as a suitable and efficient system for transient expression using *Agrobacterium tumefaciens*. Mol Plant. 2017; 10: 353-356.
85. Liu T, Cao L, Cheng Y, Ji J, Wei Y, Wang C, et al. MKK4/5-MPK3/6 cascade regulates *Agrobacterium*-mediated transformation by modulating plant immunity in *Arabidopsis*. Front Plant Sci. 2021; 12: 731690.
86. Parveez GA, Majid NA, Zainal A, Rasid OA. Determination of minimal inhibitory concentration of selection agents for selecting transformed immature embryos of oil palm. Asia Pac J Mol Biol Biotechnol. 2007; 15: 133-146.

87. Meng ZH, Liang AH, Yang WC. Effects of hygromycin on cotton cultures and its application in *Agrobacterium*-mediated cotton transformation. In Vitro Cell Dev Biol Plant. 2007; 43: 111-118.
88. Gordon-Kamm B, Sardesai N, Arling M, Lowe K, Hoerster G, Betts S, et al. Using morphogenic genes to improve recovery and regeneration of transgenic plants. Plants. 2019; 8: 38.
89. Boutilier K, Offringa R, Sharma VK, Kieft H, Ouellet T, Zhang L, et al. Ectopic expression of *Baby Boom* triggers a conversion from vegetative to embryonic growth. Plant Cell. 2002; 14: 1737-1749.
90. Srinivasan C, Liu Z, Heidmann I, Supena ED, Fukuoka H, Joosen R, et al. Heterologous expression of the *Baby Boom* AP2/ERF transcription factor enhances the regeneration capacity of tobacco (*Nicotiana tabacum* L.). Planta. 2007; 225: 341-351.
91. Heidmann I, De Lange B, Lambalk J, Angenent GC, Boutilier K. Efficient sweet pepper transformation mediated by the *Baby Boom* transcription factor. Plant Cell Rep. 2011; 30: 1107-1115.
92. Lowe K, Wu E, Wang N, Hoerster G, Hastings C, Cho MJ, et al. Morphogenic regulators *Baby Boom* and *WUSCHEL* improve monocot transformation. Plant Cell. 2016; 28: 1998-2015.
93. Laux T, Mayer KF, Berger J, Jürgens G. The *WUSCHEL* gene is required for shoot and floral meristem integrity in *Arabidopsis*. Development. 1996; 122: 87-96.
94. Xu N, Kang M, Zobrist JD, Wang K, Fei SZ. Genetic transformation of recalcitrant upland switchgrass using morphogenic genes. Front Plant Sci. 2022; 12: 781565.
95. Zhang Y, Patankar H, Aljedaani F, Blilou I. A framework for date palm (*Phoenix dactylifera* L.) tissue regeneration and stable transformation. Physiol Plant. 2024; 176: e14189.
96. Lee K, Wang K. Strategies for genotype-flexible plant transformation. Curr Opin Biotechnol. 2023; 79: 102848.
97. Wang N, Ryan L, Sardesai N, Wu E, Lenderts B, Lowe K, et al. Leaf transformation for efficient random integration and targeted genome modification in maize and sorghum. Nat Plants. 2023; 9: 255-270.
98. Kong J, Martin-Ortigosa S, Finer J, Orchard N, Gunadi A, Batts LA, et al. Overexpression of the transcription factor *Growth-regulating Factor5* improves transformation of dicot and monocot species. Front Plant Sci. 2020; 11: 572319.
99. Debernardi JM, Tricoli DM, Ercoli MF, Hayta S, Ronald P, Palatnik JF, et al. A GRF-gif chimeric protein improves the regeneration efficiency of transgenic plants. Nat Biotechnol. 2020; 38: 1274-1279.
100. Biswal AK, Hernandez LR, Castillo AI, Debernardi JM, Dhugga KS. An efficient transformation method for genome editing of elite bread wheat cultivars. Front Plant Sci. 2023; 14: 1135047.
101. Qiu F, Xing S, Xue C, Liu J, Chen K, Chai T, et ak. Transient expression of a TaGRF4-TaGIF1 complex stimulates wheat regeneration and improves genome editing. Sci China Life Sci. 2022; 65: 731-738.
102. Yang Z, Zhao M, Zhang X, Gu L, Li J, Ming F, et al. *MIR396-GRF/GIF* enhances *in planta* shoot regeneration of *Dendrobium catenatum*. BMC Genomics. 2024; 25: 543.
103. Erpen-Dalla CL, Mahmoud LM, Moraes TS, Mou Z, Grosser JW, Dutt M. Development of improved fruit, vegetable, and ornamental crops using the CRISPR/Cas9 genome editing technique. Plants. 2019; 8: 601.

104. Bahariah B, Masani MY, Jamaludin N, Fizree MP, Syuhada WS, Rasid OA, et al. Application of CRISPR/Cas9-mediated genome editing for trait improvement in oil palm. In: Industrial crop plants. Singapore: Springer Nature Singapore; 2024. pp. 201-226.
105. Masani MY, Norfaezah J, Bahariah B, Fizree MP, Sulaiman WN, Shaharuddin NA, et al. Towards DNA-free CRISPR/Cas9 genome editing for sustainable oil palm improvement. 3 Biotech. 2024; 14: 166.
106. Meijaard E, Brooks TM, Carlson KM, Slade EM, Garcia-Ulloa J, Gaveau DL, et al. The environmental impacts of palm oil in context. Nat Plants. 2020; 6: 1418-1426.
107. Gasni D, Fikri I, Latif M. Tribological study of addition oleic acid in palm and coconut oils as bio-lubricants. Mater Sci Forum. 2020; 1000: 210-219.
108. Sambanthamurthi R, Sundram K, Tan YA. Chemistry and biochemistry of palm oil. Prog Lipid Res. 2000; 39: 507-558.
109. Do PT, Nguyen CX, Bui HT, Tran LT, Stacey G, Gillman JD, et al. Demonstration of highly efficient dual gRNA CRISPR/Cas9 editing of the homeologous *GmFAD2-1A* and *GmFAD2-1B* genes to yield a high oleic, low linoleic and α-linolenic acid phenotype in soybean. BMC Plant Biol. 2019; 19: 311.
110. Jarvis BA, Romsdahl TB, McGinn MG, Nazarenus TJ, Cahoon EB, Chapman KD, et al. CRISPR/Cas9-induced *fad2* and *rod1* mutations stacked with *fae1* confer high oleic acid seed oil in pennycress (*Thlaspi arvense* L.). Front Plant Sci. 2021; 12: 652319.
111. Han Y, Teng K, Nawaz G, Feng X, Usman B, Wang X, et al. Generation of semi-dwarf rice (*Oryza sativa* L.) lines by CRISPR/Cas9-directed mutagenesis of *OsGA20ox2* and proteomic analysis of unveiled changes caused by mutations. 3 Biotech. 2019; 9: 387.
112. Wang Q, Gao H, Liu K, Wang H, Zhang F, Wei L, et al. CRISPR/Cas9-mediated enhancement of semi-dwarf glutinous traits in elite Xiang dao wan rice (*Oryza sativa* L.): Targeting SD1 and *Wx* genes for yield and quality improvement. Front Plant Sci. 2024; 15: 1333191.
113. Zheng M, Zhang L, Tang M, Liu J, Liu H, Yang H, et al. Knockout of two *BnaMAX1* homologs by CRISPR/Cas9-targeted mutagenesis improves plant architecture and increases yield in rapeseed (*Brassica napus* L.). Plant Biotechnol J. 2020; 18: 644-654.
114. Zhang J, Zhang X, Chen R, Yang L, Fan K, Liu Y, et al. Generation of transgene-free semidwarf maize plants by gene editing of *Gibberellin-oxidase20-3* using CRISPR/Cas9. Front Plant Sci. 2020; 11: 1048.
115. Sun Y, Zhou K, Wang X, Li X, Zhang X, Han N, et al. Identification and characterization of *CsERECTA*, a major gene controlling stem elongation through regulating GA biosynthesis in cucumber. Theor Appl Genet. 2024; 137: 151.
116. Murphy DJ, Goggin K, Paterson RR. Oil palm in the 2020s and beyond: Challenges and solutions. CABI Agric Biosci. 2021; 2: 39.
117. Azni IN, Namasivayam P, Ling HC, Alwee SS, Abd Manaf MA. Analysis of gene expression by EST from SSH library in dwarf oil palm. J Oil Palm Res. 2017; 29: 197-217.
118. Ting NC, Chan PL, Buntjer J, Ordway JM, Wischmeyer C, Ooi LC, et al. High-resolution genetic linkage map and height-related QTLs in an oil palm (*Elaeis guineensis*) family planted across multiple sites. Physiol Mol Biol Plants. 2023; 29: 1301-1318.
119. Zubaidah R, Said ZS. Exploiting gibberellin in oil palm height control: Strategies and way forward. Trans Malays Soc Plant Physiol. 2022; 29: 208-213.

120. Halim MA. Functional characterization of gibberellic acid related genes from oil palm in *Arabidopsis thaliana* for potential role height regulation. Serdang, Malaysia: Universiti Putra Malaysia; 2018.
121. Xiang X, Yang H, Yuan X, Dong X, Mai S, Zhang Q, et al. CRISPR/Cas9-mediated editing of *GmDWF1* brassinosteroid biosynthetic gene induces dwarfism in soybean. Plant Cell Rep. 2024; 43: 116.
122. Yarra R, Jin L, Zhao Z, Cao H. Progress in tissue culture and genetic transformation of oil palm: An overview. Int J Mol Sci. 2019; 20: 5353.
123. Singh R, Low ET, Ooi LC, Ong-Abdullah M, Nookiah R, Ting NC, et al. The oil palm *Virescens* gene controls fruit colour and encodes a R2R3-MYB. Nat Commun. 2014; 5: 4106.
124. Rao V, Chang KC. Breeding *virescens* oil palm. J Oil Palm Res. 2021; 33: 565-576.
125. Aprilyanto V, Darmawan C, Utomo C, Liwang T. Development of CRISPR/Cas9 plasmid for multiple sites genome editing in oil palm (*Elaeis guineensis* Jacq.). AIP Conf Proc. 2019; 2099: 020002.
126. Li X, Wang Y, Chen S, Tian H, Fu D, Zhu B, et al. Lycopene is enriched in tomato fruit by CRISPR/Cas9-mediated multiplex genome editing. Front Plant Sci. 2018; 9: 559.
127. Yang TX, Deng L, Zhao W, Zhang R, Jiang HL, Ye ZB, et al. Rapid breeding of pink-fruited tomato hybrids using the CRISPR/Cas9 system. J Genet Genomics. 2019; 46: 505-508.
128. Yang T, Ali M, Lin L, Li P, He H, Zhu Q, et al. Recoloring tomato fruit by CRISPR/Cas9-mediated multiplex gene editing. Hortic Res. 2023; 10: uhac214.
129. Wei L, John Martin JJ, Zhang H, Zhang R, Cao H. Problems and prospects of improving abiotic stress tolerance and pathogen resistance of oil palm. Plants. 2021; 10: 2622.
130. John Martin JJ, Yarra R, Wei L, Cao H. Oil palm breeding in the modern era: Challenges and opportunities. Plants. 2022; 11: 1395.
131. Hualkasin W, Thongin W, Petsean K, Nakkaew AP. Molecular cloning and characterization of the late embryogenesis abundant group 4 (*EgLEA4*) gene from oil palm (*Elaeis guineensis* Jacq). Songklanakarin J Sci Technol. 2013; 35: 275-285.
132. Li X, Xu S, Fuhrmann-Aoyagi MB, Yuan S, Iwama T, Kobayashi M, et al. CRISPR/Cas9 technique for temperature, drought, and salinity stress responses. Curr Issues Mol Biol. 2022; 44: 2664-2682.
133. Ahmed AI, Khan AI, Negm MA, Iqbal R, Azhar MT, Khan SH, et al. Enhancing cotton resilience to challenging climates through genetic modifications. J Cotton Res. 2024; 7: 10.
134. Zeng Y, Wen J, Zhao W, Wang Q, Huang W. Rational improvement of rice yield and cold tolerance by editing the three genes *OsPIN5b, GS3*, and *OsMYB30* with the CRISPR-Cas9 system. Front Plant Sci. 2020; 10: 1663.
135. Abdallah NA, Elsharawy H, Abulela HA, Thilmony R, Abdelhadi AA, Elarabi NI. Multiplex CRISPR/Cas9-mediated genome editing to address drought tolerance in wheat. GM Crops Food. 2025; 16: 1-17.
136. Yang Y, Li A, Liu Y, Shu J, Wang J, Guo Y, et al. *ZmASR1* negatively regulates drought stress tolerance in maize. Plant Physiol Biochem. 2024; 211: 108684.
137. Paterson RR. Oil palm survival under climate change in Kalimantan and alternative SE Asian palm oil countries with future basal stem rot assessments. For Pathol. 2020; 50: e12604.
138. Torres GA, Sarria GA, Martinez G, Varon F, Drenth A, Guest DI. Bud rot caused by *Phytophthora palmivora*: A destructive emerging disease of oil palm. Phytopathology. 2016; 106: 320-329.

139. Drenth A, Guest D. *Phytophthora palmivora* in tropical tree crops. In: *Phytophthora*: A global perspective. Wallingford, UK: CABI; 2013. pp. 187-196.
140. Misman N, Samsulrizal NH, Noh AL, Wahab MA, Ahmad K, Ahmad Azmi NS. Host range and control strategies of *Phytophthora palmivora* in Southeast Asia perennial crops. Pertanika J Trop Agric Sci. 2022; 45: 991-1019.
141. Zaidi SS, Mukhtar MS, Mansoor S. Genome editing: Targeting susceptibility genes for plant disease resistance. Trend Biotechnol. 2018; 36: 898-906.
142. Manzoor S, Nabi SU, Rather TR, Gani G, Mir ZA, Wani AW, et al. Advancing crop disease resistance through genome editing: A promising approach for enhancing agricultural production. Front Genome Ed. 2024; 6: 1399051.
143. Zaidi SS, Mahas A, Vanderschuren H, Mahfouz MM. Engineering crops of the future: CRISPR approaches to develop climate-resilient and disease-resistant plants. Genome Biol. 2020; 21: 289.
144. Wang F, Wang C, Liu P, Lei C, Hao W, Gao Y, et al. Enhanced rice blast resistance by CRISPR/Cas9-targeted mutagenesis of the ERF transcription factor gene *OsERF922*. PLoS One. 2016; 11: e0154027.
145. Liu S, Zhang F, Su J, Fang A, Tian B, Yu Y, et al. CRISPR-targeted mutagenesis of mitogen-activated protein kinase phosphatase 1 improves both immunity and yield in wheat. Plant Biotechnol J. 2024; 22: 1929-1941.
146. Fister AS, Landherr L, Maximova SN, Guiltinan MJ. Transient expression of CRISPR/Cas9 machinery targeting *TcNPR3* enhances defense response in *Theobroma cacao*. Front Plant Sci. 2018; 9: 268.
147. Li J, Deng F, Wang H, Qiang X, Meng Y, Shan W. The Raf-like kinase Raf36 negatively regulates plant resistance against the oomycete pathogen *Phytophthora parasitica* by targeting MKK2. Mol Plant Pathol. 2022; 23: 530-542.
148. Liu T, Ji J, Cheng Y, Zhang S, Wang Z, Duan K, et al. CRISPR/Cas9-mediated editing of *GmTAP1* confers enhanced resistance to *Phytophthora sojae* in soybean. J Integr Plant Biol. 2023; 65: 1609-1612.
149. Komal J, Desai HR, Samal I, Mastinu A, Patel RD, Kumar PD, et al. Unveiling the genetic symphony: Harnessing CRISPR-Cas genome editing for effective insect pest management. Plants. 2023; 12: 3961.
150. Tyagi S, Kesiraju K, Saakre M, Rathinam M, Raman V, Pattanayak D, et al. Genome editing for resistance to insect pests: An emerging tool for crop improvement. ACS Omega. 2020; 5: 20674-20683.
151. Zhang Y, Guo W, Chen L, Shen X, Yang H, Fang Y, et al. CRISPR/Cas9-mediated targeted mutagenesis of *GmUGT* enhanced soybean resistance against leaf-chewing insects through flavonoids biosynthesis. Front Plant Sci. 2022; 13: 802716.
152. Chen L, Feng L, Liang X, Li J, Liao G, Zhu L, et al. Characterizing the mechanism of serotonin alleviates rice resistance to brown planthopper *Nilaparvata lugens* (Homoptera: Delphacidae) Nymphs. Agronomy. 2022; 12: 3191.
153. Pérez-Llorca M, Muñoz P, Müller M, Munné-Bosch S. Biosynthesis, metabolism and function of auxin, salicylic acid and melatonin in climacteric and non-climacteric fruits. Front Plant Sci. 2019; 10: 136.

154. Lu HP, Luo T, Fu HW, Wang L, Tan YY, Huang JZ, et al. Resistance of rice to insect pests mediated by suppression of serotonin biosynthesis. Nat Plants. 2018; 4: 338-344.
155. Wang L, Lu H, Zhang X, He Y, Zhang J, Guo X, et al. Disruption of serotonin biosynthesis increases resistance to striped stem borer without changing innate defense response in rice. J Pineal Res. 2023; 75: e12895.
156. Murata K, Kitano T, Yoshimoto R, Takata R, Ube N, Ueno K, et al. Natural variation in the expression and catalytic activity of a naringenin 7-*O*-methyltransferase influences antifungal defenses in diverse rice cultivars. Plant J. 2020; 101: 1103-1117.