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A review of the applications of CRISPR/Cas systems in crops and postharvest losses of agricultural produce

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Abstract

CRISPR/Cas systems are the third-generation genome editing systems, which appeared in 2012 and quickly became a superstar in genome editing tools because of their great simplicity and usability compared to with ZFN and TALEN. CRISPR/Cas was originally identified as an effective acquired immune system in bacteria against virus infection and relies on RNA–DNA binding to achieve sequence specificity in genome editing. CRISPR/Cas9 system has become widely used in plants for characterizing gene function and crop improvement. Crops such as tomato, rice, banana and wheat are excellent model plants for biological research and are most important applied plants for genome editing. Genome editing has also been applied in plant breeding for improving fruit yield and quality, increasing stress resistance, accelerating the domestication of wild tomato, and recently customizing tomato cultivars for urban agriculture. In addition, genome editing is continuously innovating, and several new genome editing systems such as the recent prime editing, a breakthrough in precise genome editing, have recently been applied in plants. In this review, the advances in applications of CRISPR/Cas systems genome editing technology to enhance specific features in plants in order to mitigate postharvest losses and wastes are summarized.

Keywords: CRISPR/Cas; Postharvest losses; Postharvest wastes; Genome editing

1. Introduction

In the current scenario, world population is increasing in geometric proportion but the food materials available are increasing in arithmetic scale. By 2050, the world population is expected to reach around ten billions; just 3 decades far away from us [1]. Increasing world population along with the adverse effect of climate change and postharvest losses have threatened the world in terms of food security. To meet the food security in global scale, the yield potential and shelf life of the crops grown should nearly be doubled and to obtain this goal, highly adaptable crop varieties to biotic and abiotic stress should be introduced urgently [2].

The development and improvement of crop varieties has been carried out using conventional breeding techniques like hybridization and mutational breeding which has improved crop production to some extent. Genetic manipulation technique has been used for few decades using physical, chemical and biological mutagenesis to study the role of genes and identifying the biological mechanisms for crop improvement. Transgenic techniques have been used further for understanding the plant biology and crop improvement. Despite using these techniques, satisfaction has never been obtained due to the presence of some negative issues [3].

In recent years, the use of sequence-specific nucleases (SSNs) has been extensively applied for precise genome editing in crop species to create breakage of double strands in the target DNA [4]. The DNA then gets repaired through either non homologous end joining (NHEJ) or homology-directed recombination (HDR) pathways; the former being the more

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common one; resulting into insertions/deletions and substitution mutations in the target DNAs respectively [5]. The genome editing methods offers a huge advantage in producing defined mutants unlike that of transgenic approaches with random insertions leading to random phenotypes. Genome edited plants also carry their edited DNA for the desired traits, which offers an additional advantage and these improved crops can be used in breeding programs and resulting varieties can be used directly with relatively lesser consumption issues and lesser regulatory procedures as compared to transgenic crops/ conventional genetically modified (GM) crops [6, 7]. This review discusses the applications of recently advanced genome editing tool CRISPR/Cas9 in improving certain features in crops that are essential to mitigate postharvest losses.

2. Postharvest loss and waste (PLW)

Postharvest waste and postharvest loss are sometimes used interchangeably; however, they can be differentiated. Postharvest loss is unintentional and it describes the incidental losses that result from events occurring from farm-to-table, such as physical damage, internal bruising, premature spoiling, and insect damage, among others whereas postharvest waste, in contrast, is intentional and describes when produce is discarded because it does not meet buyer expectations, even though it is edible [8]. Produce may be rejected by growers, distributors, processing companies, retailers, and consumers for failing to meet desired or established preferences. In the US, it is estimated that 7% of postharvest losses of fruit and vegetables occur on the farm, while more than twice that is, 17% and 18% are wasted in consumer-facing businesses and in homes, respectively. Produce postharvest loss and waste (PLW) threatens environmental sustainability, and is especially catastrophic when viewed in the light of the twin challenges of global climate change and increasing population growth [9]. PLW means inefficient use of financial investments in horticulture and more critically, non-renewable natural resources. Technological measures to curb PLW, such as maintaining a cold-chain and use of plastic packaging, additionally have energy and carbon costs. Improving the shelf-life and quality attributes of postharvest crops by genetic modification or smart breeding could be among many solutions to lessen the severity of these problems.

2.1. Causes of postharvest losses

2.1.1. Primary causes

Agricultural commodities like food grains are when grown and transported to consumers are subjected to various agricultural operations like harvesting, threshing, milling, packing, storage etc. The large number of losses may occur when harvesting is not done at adequate moisture content and time of harvesting. Delayed harvesting or too early harvesting may subject the crop severe losses by various factors like attack of birds, rodents, microbes and natural calamities [10]. Threshing and cleaning are usually done to separate grains from panicles. However, threshing losses can occur in case of seed splitting, incomplete separation of seed from source, seed cracking due to excessive force. In order to prolong the storage of food grains, it is necessary to store the commodities at safer moisture content. Improper drying result in microbe growth in grains and is not desirable for storage and grinding operations. Thus, drying is an important postharvest technology in order to improve quality, prevention from insects and rodents and for transportation [11]. Lack of proper transportation facilities may result in loss of commodities to greater extent. However, the problems of loss due to transportation are relatively less in developed countries due to proper roads, infrastructures and processing equipment.

2.1.2. Biological causes

Respiration

Respiration is a physiochemical process in which stored organic materials like carbohydrate, lipids, fats are catabolized into simple compounds in order to liberate energy essential for metabolic processes. Respiration is an important phenomenon which influences the physiological and biochemical activities of horticultural produces like fruits and vegetables. In other words, the deterioration of horticultural products is directly related to respiration rate [12]. The respiration rate of horticultural produce can be estimated in terms of O_2 consumed or CO_2 evolved in various processes like development, maturation, ripening etc.

Transpiration

Transpiration is the physiological process which involves loss of water in the form of vapor from living tissues of the plant. The extreme loss of water from harvested produce is the major cause of deterioration which compromises its quality, nutrition, palatability and demand among consumers [12]. Further, the transpiration loss can be ameliorated in storage conditions by:

- Reducing air movement
- Lowering air temperature
- Raising relative humidity
- Use of protective cover like waxing and protective methods like modified atmospheric packaging, polyethylene films.

2.1.3. Microbes

Stored agricultural produce are often subjected to postharvest diseases caused by bacteria, mould, fungi and incidence of insect pest and rodents. The common pathogen that infects the postharvest produce includes *Penicillum sp.*, *Botrytis sp., Fusarium sp., Phytophthora infestans etc.* Mechanical damages and bruising during harvesting and other agricultural operations are common points of entry of pathogenic microorganisms which affect the quality and quantity of produce adversely and reduce marketability as well [12].

2.1.4. Ethylene

Ethylene is a gaseous hormone which plays an active and important role in postharvest technology of agricultural produce. It is a Ripening hormone which controls ripening process in fruits and vegetables [12]. However, it has also some undesirable effects on fruits like premature ripening, skin damage etc.

2.1.5. Environmental causes

Temperature

Temperature is one of the most crucial environment factor that affect the postharvest life of stored product. Generally, for every 100C increase in temperature, the rate of deterioration of produce increases to 2-3 folds [13]. High temperature increases transpiration rate, thus increasing water loss whereas low temperature favours microbe development. Undesirable temperature in storage conditions may cause chilling and freezing injury, heat injury which drastically affects the quality of postharvest produce.

Relative humidity

Freshly harvested fruits and vegetable possess 80-95% water by weight. The loss of humidity from horticultural produce is purely dependent on vapour pressure deficit between the surrounding air. The relative humidity is highly influenced by transpiration and respiration processes. In the meantime, where high relative humidity reduces the chances of water loss from produce it also harbors the pathogenic postharvest microorganisms as well [12].

Atmospheric condition

The composition of gaseous mixture mainly oxygen and carbondioxide plays an important role in controlling the quality of post harvested produce as it controls respiration, temperature, ethylene concentration etc. Therefore, it is necessary to regulate the gaseous composition around produce in order to reduce respiration and enhance shelf-life of produce [14]. Meanwhile the reduction of oxygen and increase of carbon dioxide in storage condition may reduce deterioration of postharvest produce. However, change in gaseous composition in storage chamber can also cause physiological disorder in produce. For example, hollow heart in potato can occur due to faulty oxygen balance and during transportation. The unbalanced gaseous composition may also cause other calamities like irregular fruit ripening, soft texture, poor skin color development etc.

Light

Light exposure may also cause some physiological change in produce along with alteration in biological process. For example, when potatoes are exposed to light, it forms green tubers due to formation of solanin and chlorophyll, which is toxic for human consumption [12].

3. Gene editing tools

When a non-specific nuclease domain is fused with sequence-specific DNA binding domain, then it is said to be engineered. Such fused nuclease has the capacity to cleave the target gene and the breaks are repaired either by NHEJ or HDR, and the whole process is given the term genome editing [15]. Naturally inspired existing technologies for genome engineering include several nucleases like Zinc finger nucleases (ZFNs), Transcription activator-like effector nucleases (TALENs) and CRISPR/Cas9 system. ZFNs and TALENs involve tedious procedure with long turn-over time, costly, and are less reliable as compared to the second generation CRISPR/Cas9 system [16]. More than $5000 per pair of commercial ZFNs [17] or TALENs makes it much more expensive. Moreover, the off target activity/toxicity and base skipping activity are higher than CRISPR/cas9. Besides this, long turn-over time has made it difficult for ZFNs and TALENs to scale up. Usually, ZFNs take several weeks to build a few pair for experts and TALENs take about a week to build a few pairs which make it more time consuming than CRISPR/Cas9 [18].

The simple, efficient and highly specific CRISPR/Cas9 system is a promising tool for genome editing and is expected to have larger impacts on plant biology and also on crop breeding. The elite cultivars can be precisely modified using genome editing technique, saving the time required compared to backcrossing in conventional breeding programs [19]. With the multiple traits being modified at a time, CRISPR/Cas9 system provides an efficient approach to pyramid breeding [20]. Gene editing by CRISPR/Cas9 has been adopted in nearly 20 crop species so far [21] for various traits including yield improvement, and biotic and abiotic stress management. NHEJ mediated gene editing is the most direct application of CRISPR/Cas9 gene editing. Biotic stress imposed by pathogenic micro-organisms account for more than 42% of potential yield loss and contributes to 15% of global declines in food production [22]. The negative regulators of disease resistance and grain development can be knocked out to obtain greater yield, host resistance against targeted pathogens and abiotic stresses like drought and salinity. In addition, CRISPR/Cas9 provides alternative approaches for delivering target genes into crops without any transgenic footprint, such as by viral infection, agro-infiltration, or preassembled Cas9 protein-sgRNA ribonucleoproteins transformation so as to bypass the traditional regulations on genetically modified organisms [23].

3.1. Processes involved in CRISPR/Cas9 technology

3.1.1. Knockout mediated crop trait improvement

Eliminating the negative traits that confer undesirable effects to the crop needs to be knocked out so as to obtain greater yield and biotic and abiotic stress tolerant crop variety. Hybrid breeding techniques and other important aspects of the crop breeding has been improved by knockout mechanism of gene editing [24].

3.1.2. Gene insertion and replacement

CRISPR/Cas9 mediated gene insertion and replacement has mainly been used for developing herbicide resistant crop varieties. Substitution of key amino acids in the conserved domains of the endogenous acetolactate synthase (*ALS*) and 5-enolpyruvylshikimate-3-phosphate synthase (*EPSPS*) can confer resistance to sulphonylurea-based herbicides [24]. Similarly, herbicide-resistant flax has been generated using a combination of single-stranded oligonucleotides and CRISPR/Cas9 [25]. Recently, glyphosate tolerance variety of cassava was generated by a promoter swap and dual amino-acid substitutions achieved at the *EPSPS* locus [26]. In maize, grain yield under drought stress condition was increased by editing *ARGOS8* gene [27]. When the *GOS2* promoter was inserted into the 5'- untranslated region of the native *ARGOS8* gene, or when it replaced the *ARGOS8* promoter, increased *ARGOS8* transcripts were detected that resulted in increased drought tolerance.

3.2. CRISPR/Cas9 applications that can mitigate postharvest lost

3.2.1. Increasing yield

To cope up with the widespread food insecurity, the major sector to be think upon is crop yield. Yield is a complex trait governed by many factors. Knocking out negative regulators known to affect yield-determining factors such as grain number, grain size, grain weight, panicle size, tiller-spreading and tiller number created the expected phenotypes in plants with loss-of-function mutations in these genes [28]. Simultaneous knockout of three grain weight–related genes in rice led to trait pyramiding, which greatly increased grain weight [29]. [30] mutated the *Gn1a, DEP1*, and *GS3* genes of the rice cultivar Zhounghua11 using CRISPR/Cas9, producing mutants with larger grain size, dense erect panicles and enhanced grain number. When Grain Weight 2 (*GW2*) gene in wheat is disrupted, it resulted in increased grain and protein content in wheat [31]. However, because most yield-related traits are quantitative and controlled by quantitative trait loci, simply knocking out individual factors may not be sufficient to increase yield in the field. [32] recently discovered a technique for large scale identification of genes that contributes to complex quantitative traits by combining pedigree analysis, whole-genome sequencing, and CRISPR/Cas9 technology [31]. The author sequenced 30 cultivars of the rice variety IR8 and selected 57 genes from high-yielding lines for gene editing via knockout or knockdown using Cas9 or dCas9. This provided insight in crop yield development and may facilitate molecular breeding in rice [33].

3.2.2. Increasing quality

With the advent of convenient CRISPR/Cas9 techniques, scientists have recently added the fragrance trait to more than 30 elite rice cultivars in major planting areas of China [24]. CRISPR/Cas9 technology has been used to target the *FAD2* gene in *Camelina sativa*, the emerging oil seed plant, to improve oleic acid content while decreasing polyunsaturated fatty acids [34]. Gluten proteins from cereal crops which trigger celiac disease are formed by α-gliadin gene family. The CRISPR/Cas9 genome editing tool offers a new way to alter traits controlled by large gene families with redundant functions. Indeed, by knocking out the most conserved domains of α-gliadin family members, researchers have created low-gluten wheat [35]. CRISPR/Cas9 has been used in rice to generate the targeted mutations in *SBEIIb*, leading to higher proportion amylose, which improved the nutritional content and fine structure of starch [36]. Waxy maize with improved digestibility and higher bio-industrial application is prepared by DuPont Pioneer by knocking out the maize waxy gene (*Wx1*), which encodes Granule-bound starch synthase (*GBSS*) gene responsible for making amylose. Development of parthenocarpic tomato fruits with huge market potential in processing industry was achieved simultaneously by two different groups. [37] carried out knockout of *Slagamous-like 6*(*SlAGL6*) gene that otherwise severely hamper fertilization-dependent fruit set; made mutant plants capable of producing parthenocarpic fruits under heat stress conditions. Alternatively, the other group has obtained parthenocarpic tomato fruits by mutating *SlIAA9* gene involved in auxin signaling pathway that suppress the parthenocarpy [38]. By knocking out the gene responsible for polyphenol oxidase enzyme synthesis using CRISPR/Cas9 developed a non-browning mushroom which triggers the postharvest quality and fetch higher prices [39].

3.2.3. Increasing biotic and abiotic stress tolerance

The yield loss caused by the disease causing pathogen and other abiotic stress is significantly higher. According to [40] the estimated yield loss due to plant pathogens is up to 16%. So many attempts have been made in molecular biology to make disease resistant varieties. [41] has simultaneously edited three homoeoalleles *TaMLOA1, TaMLOB1* and *TaMLOD1* that confer heritable resistance to powdery mildew. The CRISPR/Cas9 technology is used to generate *Taedr1* wheat plants by simultaneous modification of the three homeologs of *EDR1* which resulted in plants resistant to powdery mildew [42]. In rice, resistant varieties against blast disease and bacterial blight were obtained separately by mutagenesis of *OsERF922, OsSEC3A* and *OsSWEET13* genes [43, 44]. Further, powdery mildew and bacterial speck resistant tomato varieties were obtained by editing *SlMLO1* and *SlJAZ2* respectively [45]. By the modification of *CsLOB1* promoter, canker symptoms were alleviated in Duncan grapefruits [46]. The technology was further used to disrupt the coding region of *CsLOB1* resulting in no canker symptoms in Duncan grapefruit [46]. [47] conducted a research in cucumber by disrupting *eIF4E* (Eukaryotic translation initiation factor 4E), broad virus resistance was developed. The plants were seen immune to cucumber Vein Yellowing virus (Ipomovirus) and were also resistant against potyviruses, Zucchini yellow mosaic virus and Papaya ring spot mosaic virus-W. CRISPR/Cas9 has also produced tungro disease resistant *eif4g* rice [48] and cotton leaf curl disease-resistant *clcud* cotton [49]. The destructive insect pests of rice that is, plant hoppers and stem borers are the major yield reducing factors in rice. It was found that disrupting the *OsCYP71A1* blocked serotonin biosynthesis and greatly increased salicylic acid levels, thereby confer resistance against these pests [50].

By developing *slnpr1* mutant from isolated *SlNPR1* gene of tomato 'Ailsa Craig' using CRISPR/Cas9 resulted into drought tolerance in tomato [51]. Modification of *OsPDS, OsMPK2, OsBADH2* genes in rice had led to increased tolerance to abiotic stresses [52]. Similarly Zhang *et al.* (2014) identified that modifications on *OsDERF1, OsPMS3, OsEPSPS, OsMSH1, OsMYB5* genes lead to the drought tolerant varieties in rice. Similarly, *OsHAK-1* and *OsPRX2* gene were edited in rice for obtaining low cesium accumulation and potassium deficiency tolerance respectively [53, 54]. CRISPR/Cas9 has been used to study the role of genes. Plant annexins which play a significant role in stress tolerance and plant development were tested using CRISPR/Cas9. Rice annexin gene (*OsAnn3*) was tested for its role in cold stress by *OsAnn3* CRISPR knockouts [55].

3.2.4. CRISPR/Cas9 for hybrid breeding

Using CRISPR/Cas-mediated gene knockout, tremendous progress has been made to produce male sterile lines. The thermosensitive genic male sterile 5 gene (*TMS5*) was knocked out in maize to generate male sterile line [56]. In similar ways thermosensitive male-sterile *tms5* lines were developed in rice [57], photosensitive genic male-sterile *csa* rice [30] and *ms45* wheat were developed [58]. Recently, haploid rice was obtained by knockout of *OsMATL* by CRISPR/Cas9 [59]. The selfish-gene suicide mechanism in rice caused by the toxic *ORF2* gene was knocked out improving the fertility of japonica-indica hybrids [60]. Similarly, CRISPR/Cas9 can be used by the breeders for domestication of wild varieties. Wild tomato accessions were introduced by targeting the coding sequences, cis-regulatory regions, and upstream open reading frames of genes associated with tomato morphology, flower and fruit production and ascorbic acid synthesis [61].

3.2.5. CRISPR/Cas9-mediated genome editing of MaACO1 (aminocyclopropane-1-carboxylate oxidase 1) promotes the shelf life of banana fruit

Banana is a fruit with high nutrient content and high economic importance. It is regarded as a main staple food in developing countries. As a typical climacteric fruit, banana will ripen and decay in one week after exogenous ethylene induction. The short shelf life of banana largely limits its storage, transportation and marketing and causes great postharvest loss [62]. The shelf life of banana is closely related to ethylene production, which is the first factor considered for developing postharvest preservation technology. A reduction of endogenous ethylene production or impaired ethylene signal transduction by genetic modification might be highly efficient methods to delay the ripening process [63]. Chunhua et al. [64] created several MaACO1-disrupted banana plants with different editing patterns using the CRISPR/Cas9 system. The mutant fruits exhibited reduced ethylene synthesis and extended shelf life under natural ripening conditions. Moreover, MaACO1- disrupted fruit was also sensitive to ethephon and ripened normally after ethephon treatment. In addition, the vegetative growth, lifecycle and fruit quality of the MaACO1-disrupted line were comparable to those of wild-type plants except for slightly lower height and yield. These data suggest that MaACO1 is an ideal target for creating fruit with a long shelf life using the CRISPR/Cas9-mediated editing system. The application of newly created germplasm will greatly reduce the postharvest losses and will increase the economic value of the banana industry by improving the shelf life of banana fruit.

4. Conclusion

Agricultural commodities after harvest are liable to decay thus, causing loss of quality and quantity. Different factors are responsible for causing postharvest losses. Ethylene as a gaseous hormone plays an important physiological role in fruit ripening as well as accelerates the rate of aging and senescence. It also acts as a signalling molecule and controls ripening process in plants. Different transgenic approaches such as CRISPR/Cas systems and breeding methods are developed in order to prevent postharvest losses. Therefore, the significance of postharvest physiology is utmost important in the field of agriculture in developing genetically modified crops.

Compliance with ethical standards

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There is no conflict of interest in the publication of this reviewed article.

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