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Accomplishments in vegetable crop improvement through CRISPR/CAS9 gene editing system

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Abstract
The demand for food security is expanding as the world's population expands gradually. It is now urgently necessary to apply cutting-edge technologies to accelerate the pace and scale of our vegetable production. One of the primary components of the global food supply chain is vegetable crops. They have evolved into staple meals in many cultures throughout the world because of their variety, flavour profile, and nutritional value. However, due to their physiology, they are more vulnerable to harm from biotic stress and climate change. Therefore, there is an urgent need for novel kinds with higher yields, greater adaptability, and tolerance to biotic and abiotic stress. Conventional breeding is difficult and, depending on the species, might take over 20 years to generate a new competitive variety since it depends on the genetic variability already existing in the genetic pool. Here is where the variety, usability, and high efficiency of technologies like CRISPR/Cas9 may produce superior outcomes. Genome editing using CRISPR/Cas9 has the potential to profoundly alter how crop development techniques are developed in the near future.

Keywords: Vegetable crops, CRISPR/Cas9 gene editing system, abiotic and biotic stress tolerance, quality enhancement, shelf life extension

Introduction
Vegetable crops are one of the most significant food sources because of their diversity. They can give critical nutrients for human nutrition, including vitamins, minerals, fibre, and metabolites. They are grown all over the world. Vegetables, on the other hand, are more susceptible to harm from unfavourable weather circumstances and climate change due to their physiology (Karkute *et al*., 2017) [25]. It is crucial to acquire new types that can swiftly adapt to these changing circumstances. For many years, traditional breeding has indeed been crucial to meet the ongoing demand for larger yields. However, this approach is reliant on inherent genetic diversity. Additionally, due to intense selection, the genetic foundation of some vegetable species has been shrinking, restricting the accessibility of alleles for further advancement (Karkute *et al*., 2017) [25]. Conventional breeding is very difficult and time-consuming; for certain species, it might take up to 20 years to create a new variety to be produced commercially.

Genetic modification is a way to get around the aforementioned restrictions. For the past 30 years, a number of methods, including particle bombardment, Agrobacterium, and direct DNA absorption, have been utilised to transfer foreign genetic material into vegetable cells (Jaganathan *et al*., 2018) [8]. These methods have been essential for understanding how genes work biologically, making them a great source for developing novel features in vegetable crops. Furthermore, public concern regarding the insertion of undesirable genetic material originating from vectors and the usage of flag genes has resulted in highly rigorous regulation across the board (Waltz, 2018) [19]. Techniques rely on site-specific nucleases that produce well-defined and targeted mutations have been effectively employed to alter numerous plant species for more than ten years. These genome editing technologies have emerged as a potent substitute for enhancing vegetable crops. Due of its simplicity, adaptability, and higher frequencies, the Clustered Regulatory Interspaced Short Palindromic Repeat Associated Protein System (CRISPR/Cas9) has replaced earlier methods including Zinc Finger Nucleases (ZFNs) and Transcriptional Activator-Like Effector Nucleases (TALENs) (Zhang *et al*., 2017) [23]. The most recent research works of this technology on vegetable crops and the implications for the future are explained in this review.

Research accomplishment in vegetable improvement for abiotic stress tolerance
By altering the SlMAPK3 gene, Wang and others (2017) [20] produced lines that were less resistant to the effects of drought stress. By choosing individuals with high levels of gene

expression, these findings identifying the conserved function may be helpful for breeding purposes.

In a subsequent study in watermelon, the Acetolactate synthase (ALS) gene was the target for editing in order to produce modified plants that are herbicide-resistant (Tian *et al*., 2018) [18]. It's interesting to note that this study tried a point mutation. Herbicide resistance may be conferred by a codon shift in this gene from C to T. In potato, In order to diminish vulnerability to herbicides that suppress ALS, the ACETOLACTATE SHYNTHASEI (ALSI) gene was targeted for alteration (Butler *et al*., 2016) [4].

In lettuce, LsNCED4 gene, which controls thermo-inhibition of seed germination, was the focus of Bertier and others (2018) [2] research. An analysis of 368 T2 and 47 T1 primary transformants revealed that this gene's deletion significantly increased the maximum temperature tolerance for seed germination, with more than 70% of seeds able to germinate at 37 °C. In lettuce, Zhang and others (2018) [24] targeted deletion of LsGGP2 that induced increased oxidation stress tolerance.

Research accomplishment in vegetable improvement for biotic stress tolerance

In order to impart tolerance to powdery mildew in tomatoes, the gene SlMlo1 was knocked out. Plant diseases and certain top cultivars' vulnerability to them are a serious worry that has to be addressed. The outcomes demonstrated that this strategy was effective in producing lines that were pathogen-resistant (Nekrasov *et al.*, 2017) [14]. A tomato cultivar called Moneymaker was created by Ortigosa and others (2019) [15] with resistance to the bacterial speck disease brought on by *Pseudomonas syringae* pv *tomato*. They concentrated on changing the SlJAZ2 gene, which led to JAZ2 variants that were truncated and lacked the C-terminal Jas domain. Although there was less bacterial infiltration through the stomata in this modified type, its necrotroph resistance was unaffected.

Cucumber was the first species in the *Curcubitaceae* family to successfully report gene editing using the CRISPR-Cas9 system (Chandrasekaran *et al*., 2016) [5]. For the establishment of widespread viral resistance, the authors focused on the elF4E gene at two distinct loci. They were successful in giving cucumbers wide viral resistance, although the procedure's effectiveness was quite low.

Research accomplishment in vegetable improvement for modified physiology/phenology/morphology

In numerous plant species, including lettuce, Woo and others (2015) [21] reported the direct transport of ribonucleoproteins (RNPs) into protoplast cells and the production of specific genomic changes. To cause mutations in the BIN2 gene, that expresses an inhibitory regulator in the brassinosteroid signalling pathway, they transfected protoplasts. After successfully regenerating whole plants, analysis showed that they were able to pass the mutant allele on to their offspring.

In carrot, Klimek and others (2018) [11] published their findings on targeted mutagenesis utilizing the CRISPR-Cas9 system for gene editing in this species. The ability to prevent the manufacture of anthocyanins was investigated using multiplexing CRISPR-Cas9 vectors harboring two single-guide RNAs (gRNAs) that target the carrot flavanone-3-hydroxylase (F3H) gene. The outcomes demonstrated that the AteCas9 system was the most efficient at producing desired mutations, with an astonishing 90% efficiency. The discoloration of calli caused by the F3H gene knockout validates the functional significance of this gene in the anthocyanin production pathway.

In tomato, editing of CLV3 and SPG5, which are involved in meristematic proliferation and flowering repression, respectively, resulted in lines with larger fruits due to aberrant meristem development and plants that bloomed earlier due to their reduced sensitivity to long days (Soyk *et al*., 2017) [16]. In Chinese cabbage, mutation on BraFLCs resulted in early flowering phenotype that was insensitive to vernalization (Jeong *et al*., 2019).

In tomato, Brooks and team (2014) [3] chose the gene SLARGONAUTE (SLAGO7) which, when modified, would result in a very different phenotype if disrupted. The findings revealed T0 plants with pronounced needle-like leaf presence. These outcomes demonstrated that the gene edition technique had once again been effective. SlAGL6 gene mutations caused by Klap *et al*., (2017) [10] led to the development of fruitless plants. In the second study, SlIAA9 was knocked out, resulting in plants with altered leaf shapes and the anticipated seedless fruit. In cucumber, Hu and others' (2017) [6] main goal was to mutate the CsWIP1 gene using CRISPR-Cas9 to create gynoecious lines. Utilizing the more potent CsU6 promoter allowed the system to be optimized. T0 mutants had a gynoecious phenotype with smaller leaves than wild-type plants and top nodes containing solely female flowers. In cabbage, Ma and others (2019) [13] mutated three genes namely BoPDS1, BoSRK3 and BoMS1, which resulted in male sterile lines.

Research accomplishment in vegetable improvement for enhanced quality and postharvest shelf life

In tomato, successful production of mutant lines has been documented with greater amounts of aminobutyric acid and lycopene (Li *et al*., 2018) [12]. In potato, Andersson and others (2017) [1] focused on nutritional value to improve starch quality by deleting the GBSS gene. In as many as 2% of the regenerated lines, they produced mutations in all four alleles in a single transfection. In Chinese Kale, Sun and others (2020) [17] produced an yellow-colored phenotype with appealing market aspects, by mutating the gene BoaCRTISO.

Ito and others (2015) [7] targeted the gene RIN and produced plants with tomato fruit that was only partially ripe. Yu and others (2017) [22] generated mutated lines through CRISPR/Cas9 system that had a prolonged shelf life. Mabli and others (2020) produced eggplants that had lowered enzymatic browning phenomenon, through mutation in the gene SmelPPO

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