

CRISPR / CAS9 Applications in Breeding and Improvement of Tomatoes, *Solanum lycopersicum* : A Review

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ABSTRACT

Tomato (*Solanum lycopersicum*) is an essential plant because of its social and economic importance. Therefore, recent researches have been focusing on improving tomato production and its quality. The introduction of clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated proteins (CRISPR/Cas9) system provides unique opportunities to better understand the gene functions and to rapidly generate new tomato cultivars harboring desired traits such as disease resistance, better harvest quality and abiotic tolerance. This review aims to provide latest information about the application of CRISPR/Cas9 system on tomato breeding. Tomato is an important source for the increasing demand for better quality and quantity for human daily consumption. As a result, tomato production is required to enhance its productivity and reduce environmental impacts. So far, a great amount of achievements have been obtained in many research. With the emergence of CRISPR/Cas9 system, tomato breeders and researchers are offered a novel tool to rapidly understand traits of great economic significance. It is hoped that CRISPR/Cas9 system will accelerate the research progress in tomato industry in the next coming decades.

KEY WORDS: CRISPR/CAS9, TOMATO PRODUCTION, TOMATO CULTIVARS, DISEASE RESISTANCE, ABIOTIC TOLERANCE.

INTRODUCTION

The tomato is a major vegetable crop that has achieved tremendous popularity over the last century. It is grown in practically every country of the world - in outdoor fields, greenhouses, and net houses (Boase, et al. 2018;

Chen, et al. 2018). The tomato plant is very versatile, and the crop can be divided into two categories; fresh market tomatoes, which we are concerned with and processing tomatoes, which are grown only outdoors for the canning industry and mechanically harvested. In both cases, world production and consumption has grown quite rapidly over the past 25 years (Wang, Tavano, et al. 2019).

Tomatoes, aside from being tasty, are very healthy as they are a good source of vitamins A and C. Vitamin A is important for bone growth, cell division and differentiation, for helping in the regulation of immune system and maintaining surface linings of eyes, respiratory, urinary and intestinal tracts (Li, Wang, et al. 2018). Vitamin C is important in forming collagen, a protein that gives structures to bones, cartilage, muscle,

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and blood vessels. It also helps maintain capillaries, bones and teeth and aids in the absorption of iron (Hu, et al. 2019).

Currently the tomato has a higher consumption rate in more developed countries and is often referred to as a luxury crop. In Israel, for example, the tomato is such an important part of the diet that it is a major part of the food basket, which is used when calculating the consumer price index. In other words, a scarcity of tomatoes can cause the Consumer Price Index to rise and influence the inflation rate (Ding, et al. 2018; Wang, Samsulrizal, et al. 2019). In developing countries, the tomato is becoming a more important part of the food basket, but the goal of the farmer is to produce quantity not quality so people can eat. As varieties improve and new cultivars with better resistance to various diseases are developed, it will become easier to grow the crops in more marginal conditions and the tomato will become a more important part of the diet in poorer countries as well (D'Ambrosio, et al. 2018; Prihatna, et al. 2018).

Genome editing technique and its principles: In the last few decades, progresses in breeding approaches, especially forward genetic approaches, have played vital roles in elucidating the molecular mechanism that control agriculturally important traits in tomato. The advantage of conventional plant breeding consists of increasing the availability of genetic resources for crop improvement through introgression of the desired traits (Wei, et al. 2013; Li, et al. 2016; Wang, Samsulrizal, et al. 2019). However, some plants are at risk of becoming susceptible to environmental stress and losing genetic diversity. Thus, traditional cultivation methods are not sufficient to resolve global food security issues (Yang, Wang, et al. 2017).

The newly developed technologies in genome-editing have overcome the limitations of traditional breeding methods in elaborating functional genomics and crop improvement in tomato. These genetic innovations provide more accurate, time-saving, efficient targeted genomic modifications, including whole-gene insertion or deletion, stacking or pyramiding of genes, in a transgene-free manner (Ran, et al. 2013; Li, Fu, et al. 2018; Romero and Gatica-Arias 2019). Gene editing is a molecular biology technique that intentionally targets user-defined DNA sites within the genome for the purpose of elucidating functions of unknown genes. Since modified genetic information in the parental lines is passed to next generations, gene editing can be employed to purposely alter traits of agricultural importance to develop new cultivars or breeding lines (Boase, et al. 2018; Li, Zhang, et al. 2018).

Various gene editing techniques have been established including zinc finger nuclease (ZFN), transcription activator-like effector nuclease (TALEN) and cluster regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) (CRISPR/Cas9) (Prihatna, et al. 2018). All these tools rely on the specificity of the endonucleases that recognize and cleave

DNA at desired sites to facilitate mutations induced by cellular repair mechanism. In this review, we aim to provide the latest updates of CRISPR/Cas9 application on swine breeding, although TALEN and ZFN can obtain the same outcomes (Dahan-Meir, et al. 2018; Wang, Tavano, et al. 2019).

ZFN and TALEN are two early gene editing techniques that employ similar conceptual nuclease structure to introduce genetic mutation. Both systems depend on the specificity of the DNA-binding domain of zinc finger protein (ZFP) in the ZFN system and transcription activator-like effector (TALE) in the TALEN system. Since each zinc finger in the ZFP recognizes every triplet on single-strand DNA, designing 3-6 zinc finger components in combination will therefore attach to 9-18 base pairs on aimed regions to achieve specificity (Forsyth, et al. 2016). On the other hand, the improved targeting property of TALE relies on the programmable tandem repeat modules, of which each module specifically binds to a single base pair. The order of the tandem repeat modules can be rearranged to obtain better directing at chosen DNA sequence. After the binding to DNA region, both ZFP and TALE will orchestrate the dimerized endonuclease Fok1 to break the double strand DNA at predetermined regions (Tomlinson, et al. 2019; Wang, Samsulrizal, et al. 2019).

The introduction of DSB generated by ZFN and TALEN will trigger the DNA repair mechanisms including non-homologous end-joining (NHEJ) or homologous recombination (HR) (Ito, et al. 2015). In the error prone NHEJ pathway, the two ends of the cleaved DNA are joined and ligated, resulting in the generation of insertion or deletion at the site of DSB, thus producing knock-out mutation (Dahan-Meir, et al. 2018). In the HR pathway, a site-directed nuclease and an exogenous DNA template harboring homologous sequence to the DSB regions are required to facilitate the insertion of single or multiple transgenes, thereby gaining knock-in mutation. Accumulation of reports have demonstrated the successful application of ZFN (Li, Wang, et al. 2018; Li, et al. 2019). The latest CRISPR/Cas9 is extensively employed in genome editing research thanks to its reliability, efficiency and simplicity (Mishra, et al. 2018). Basically, CRISPR/Cas9 is a RNA-mediated adaptive immune system that can be found in bacteria, and archaea (Xie and Yang 2013). This immune protection provides resistance against genetic attacks and later stores infection histories in a form of spacer sequences for future safety.

These spacers function in concert with Cas9 endonuclease proteins to monitor, recognize and degrade exogenous DNA. This process can be divided in three stages: spacer acquisition, biogenesis, and immunity. In the spacer acquisition stage, the foreign DNA is identified, captured, and embedded into the CRISPR locus in a form of spacer. Subsequently, the expression of the CRISPR/Cas9 system will be initiated in the biogenesis stage, in which the primary CRISPR-RNAs (crRNAs) is synthesized from the CRISPR locus and subsequently undergone many

processes to become crRNAs. Finally, in the immunity stage, the crRNAs, together with the trans-activating RNAs (tracrRNAs), will associate with Cas9 endonuclease, forming a ribonucleotide complex. This complex will

initiate interference and consequent degradation of the targeted foreign DNA by base pairing recognition mechanism and endonucleases, respectively.

Table 1. The application of CRISPR/Cas9 in tomato breeding improvement

Target gene	Gene function or phenotype	Classification of targeted gene	References
SIALS1, SIALS2	Herbicide resistance	Abiotic stress	(Danilo, et al. 2019)
SIJAZ2	Bacterial speck resistance	Biotic stress	(Ortigosa, et al. 2019)
APETALA2, NONRIPENING FRUITFUL	Fruit development and ripening	Harvest quality	(Wang, Tavano, et al. 2019)
PECTATE LYASE POLYGALACTURONASE 2A BETA GALACTANASE	Fruit color and firmness	Harvest quality	(Wang, Samsulrizal, et al. 2019)
SINPR1	Drought tolerance	Abiotic stress	(Li, et al. 2019)
CBF1	Chilling tolerance	Abiotic stress	(Li, Zhang, et al. 2018)
SIGRAS8	Fruit development	Harvest quality	(Hu, et al. 2019)
Solyc08075770	Fusarium susceptibility	Biotic stress	(Prihatna, et al. 2018)
lncRNA1459	Fruit ripening	Harvest quality	(Li, Fu, et al. 2018)
SIDML2 COAT PROTEIN	Fruit ripening	Harvest quality	(Lang, et al. 2017)
REPLICASE FROM TYLCV	Viral resistance	Biotic stress	(Tashkandi, et al. 2018)
RIN OVATE FASCIATED FRUIT WEIGHT 2.2	Fruit ripening Fruit shape Fruit size	Harvest quality	(Jung, et al. 2018)
MULTIFLORA	Fruit number	Harvest quality	(Zsögön, et al. 2018)
SIORRM4	Fruit ripening	Harvest quality	(Yang, Zhu, et al. 2017)
SIMAPK3	Drought tolerance	Abiotic stress	(Wang, et al. 2017)
PSY	Fruit color	Harvest quality	(Filler Hayut, et al. 2017)
SIMlo1 Coilin gene	Powdery mildew resistance Viral resistance	Biotic stress	(Nekrasov, et al. 2017)
	Osmotic and salt tolerance	Abiotic and biotic stress	(Makhotenko, et al. 2019)
StALS1, StALS2	Herbicide resistance	Abiotic stress	(Veillet, et al. 2019)
Fascilin-like arabinogalatan protein	Root hair development under phosphorus stress	Abiotic stress	(Kirchner, et al. 2018)
eBSV	Viral resistance	Biotic stress	(Tripathi, et al. 2019)

It was not until the work of Jinek et al. (2012), the significant contribution of CRISPR/Cas9 technology to genome editing begins to emerge by the establishment of the programmable version of CRISPR/Cas9 (Jinek, et al. 2012). This modified version of CRISPR/Cas9 is made up of the customizable single strand RNA (sgRNA), which is the fusion product of crRNA and tracrRNA, the recombinant Cas9 protein and. This combination will result in Cas9/sgRNA complex that targets and initiates DSB at specific DNA sequences. Once DSBs are introduced, NHEJ or HDR strategy is activated to repair

the DNA damages, leading to gene knockout, or gene knock-in, respectively (Xu, et al. 2017; Yang, Wang, et al. 2017). CRISPR/Cas9 system has been widely employed in various research model research, including Prokaryotes (*Escherichia coli*) (Jiang, et al. 2015) and *Eukaryotes* (*Saccharomyces cerevisiae*, *Drosophila melanogaster*, *Caenorhabditis elegans*, *Arabidopsis thaliana*, etc.) (Gratz, et al. 2015; Dickinson and Goldstein 2016; Miki, et al. 2018; Laughery and Wyrick 2019). CRISPR/Cas9 has revolutionized the tomato breeding having far reaching effects on its quality improvement.

CONCLUSION

Tomato is an important source for the increasing demand for better quality and quantity for human daily consumption. As a result, tomato production is required to enhance its productivity and reduce environmental impacts. So far, a great amount of achievements have been obtained in many research. With the emergence of CRISPR/Cas9 system, tomato breeders and researchers are offered a novel tool to rapidly understand traits of great economic significance. It is hoped that CRISPR/Cas9 system will accelerate the research progress in tomato industry in the next coming decades.

Conflict of Interest: The authors declared that present study was performed in absence of any conflict of interest.

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