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CRISPR-Cas: A Potential Genome Editing Tool in Crop Improvement

Mamatha Bhanu L S

Assistant Professor, Department of Biotechnology, Yuvaraja's College, University of Mysore, Mysuru, Karnataka, India, ls.mamatha@gmail.com

Abstract: An innovative method, the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 gene editing system, has significantly revolutionized agriculture by improving the quality of crops and sustaining the environment. CRISPR technology is based on the natural defense mechanism that bacteria and archaea have adapted against invading viruses or other foreign DNA. A similar mechanism is utilized in a genome engineering tool that has exceptional crop breeding progression by virtue of its accuracy in particular gene editing. This study outlines the present application of CRISPR/Cas9 technology in agricultural crop yield, quality, texture modulation, palatability, nutritional components, disease resistance, and environmental stress. In plants, CRISPR/Cas9 gene-editing includes the selection of specific target sites, single guide (sgRNA) design and synthesis, ribonucleoprotein (RNP) or transformation carrier delivery in plant cells, and gene-edited plant transformation and regeneration. The knockout of three *mlo* genes in wheat confers wheat resistance to powdery mildew disease. CRISPR/Cas9 system, knockout gene Clpsk1 that encodes phytosulfokine (PSK), a precursor disulfated pentapeptide plant hormone, results show that watermelon with significant enhanced plant resistance to *Fusarium wilt* disease and regulate plant immunity. Knockout of gene *ppa6* enhanced rice tolerance to alkaline stress. Furthermore, simultaneous editing of multiple genes has contributed to pathway-level plant biotechnology research that widely expands genome engineering of agronomic traits and its adoptability. All CRISPR/Cas systems require a specific PAM sequence, which guides the editing sites with specificity. Consequently, developing a PAM-independent CRISPR/Cas system, exploring new cas proteins, and modifying Cas enzymes for expanding PAM variants will boost the application of CRISPR/Cas in applied research in agriculture, precision breeding, and ensuring food security.

Keywords: Genome editing; CRISPR; Cas9; Agriculture; Crop improvement

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1. Introduction:

At present, agricultural food production system is facing the difficulty due to climate change, environmental stress leading to decrease in grain quality and crop yield. Crop yield and quality is of utmost significance to provide nutritional security to mankind, the current scenario of food security is challenging with the growing population and extreme climatic fluctuations (Jaganathan et al., 2018). Human population is estimated to reach nearly 10 million by 2025, to cope up with global zero hunger, a sustainable increase in food production by around 60-100% is needed (FAOSTAT, 2016). World food production and its distribution depends on farmers, breeders as well as policy makers and the government to by adapting scientific approaches to ensure food security and eliminate hunger (Fiaz et al., 2021). Traditional breeding techniques are not enough to meet the growing populations so, recent effective, genome editing techniques are employed. Genome editing (GE) is a novel technique that manipulates the plant genome by

deletion and insertions of single-nucleotide or large fragment substitution which is heritable (Gaj et al., 2013). However, genome editing has importance in agronomic quality traits of many monocots and dicots that can mitigate environmental stress, climatic fluctuations, and higher yield with nutritional quality (Matres et al., 2021). Genome editing have sequence-specific nucleases that targets the DNA at specific site and create double-stranded breaks (DSB), this breaks are repaired through (NHEJ) non-homologous end joining or (HDR) homologous-directed recombination pathways producing insertion, deletions (INDEL) or substitution of base in the target region of DNA ((Jinek et al., 2012). Gene edited crops are of much use now days for breeding new varieties as there is no marketing and consumption issues. Genetically modified crops have an ethical issue which is less compared with genome edited crops (Waltz, 2018). The oldest genome editing developed in 1900s commonly known as first generation genome editing technologies include zinc finger nucleases (ZFNs) that attach to specific sequences in DNA zinc finger motifs and breaks are made on the double stranded indefnite domain of FoK1 endonuclease (Pabo et al., 2001). This has been advantageous in many plants like, maize, soyabean and tobacco (Ainley et al., 2013; Baltes et al., 2014). Transcription activator-like effector nucleases (TALENs), a substitute for ZFNs, they are naturally occurring extended segments of transcription activator-like effector (TALE) sequences attached to the Fokl domain with TALE repeat arrays (Christian et al., 2010). They are advantages over ZFNs and is used to initiate non-homologous mutations in plants (Joung & Sander, 2012) and used in rice (Li et al., 2012), tobacco (Zhang et al., 2013) and Arabidopsis (Cermak et al., 2011). In this regard, the up gradation of genome editing technologies has greater impact on research in plant breeding by the Clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9, second generation gene editing technique being effectively applied in multiple crop plants (Ansari et al., 2020).

2. CRISPR/Cas9 Gene-Editing technology

Escherichia coli was the first model organism that reported Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system and later in archaea (Ishino et al., 1987; Mojica et al., 1993) over 30 years ago. Cas proteins were found to be associated with CRISPR was originally known to perform DNA repair (Jansen et al., 2002; Makarova et al., 2002) which constitute an adaptive immune system which is RNA-guided regulated by CRISPR RNA (crRNA) with a set of Cas proteins (Class 1) or a multi-domain Cas protein (Class II). Based on a particular protein that cleaves specific DNA, the two classes of Cas proteins are divided into three types; In the class 1 CRISPR-Cas systems, the effector module consists of a multi-protein complex in the effector module with three types I, III and IV whereas class 2 systems has single effector protein with II, V and VI types (Makarova et al., 2015). Furthermore, based on the CRISPR-Cas locus architecture there are many subtypes. Makarova et al., (2020) reported two classes of CRISPR-Cas, six types and thirty three subtypes.

2.1. Mechanisms of CRISPR/Cas9

The CRISPR-Cas9 recognizes and cleaves foreign DNA or RNA segment in sequence-specific manner. This is an adaptive defense mechanism in prokaryotes which can be divided into three stages: (i) Spacer acquisition/ Adaptation, (ii) Biogenesis of crRNA, and (iii) Target interference (Hille & Charpentier, 2016). Spacer acquisition/ Adaptation - the sequence of mobile genetic elements, protospacer is included into the CRISPR array yielding a new spacer. This process allows to memorize the foreign DNA/RNA by the host organism and then they are transcribed into long precursor CRISPR RNA (pre-crRNA) by the two proteins, Cas1 and Cas2 (Babu et al., 2011). The spacer acquisition event is processed by Cas6 protein in type I and III systems. In type II CRISPR-Cas systems, crRNA maturation requires tracrRNA, RNase III and Cas9 protein, In type II-A CRISPR-Cas systems, the (protospacer adjacent motif) PAM-recognizing domain of Cas9 is involved in protospacer selection (Wei et al., 2015). Later, Cas9 recruits other protein, Cas1, Cas2 and possibly Csn2 for integration of the new spacer into

the CRISPR array which is conserved among all class II CRISPR-Cas systems (Silas et al., 2016). Biogenesis of crRNA - after adapatation, CRISPR array is transcribed into a long precursor crRNA (pre-crRNA) that is again processed into mature guide crRNAs containing the memorized foreign sequences (Carte et al., 2008). In type I and III Cas6 protein carryout the processing step to obtain intermediate species of crRNAs that are flanked by a short 5′ tag. In type II systems, tracrRNA carry out the processing of the pre-crRNA. The anti-repeat sequence of this RNA forms an RNA duplex with every repeats of the pre-crRNA, by Cas9. The duplex is then cleaved by RNase III forming an intermediate form of crRNA that undergoes further maturation and lead to the mature small guide RNA (Deltcheva et al., 2011). Interference - is the last step of defence, crRNAs that are matured guides to interfere with the invading nucleic acids specifically. Class 1 systems engage Cascade (CRISPR-associated complex for antiviral defence)-like Cas3 complexes to achieve target degradation, while in class 2 systems, a single effector protein is sufficient for target interference, tracrRNA:crRNA duplex guides the effector protein Cas9 to creates a break in the target double stranded DNA (Deveau et al., 2008; Marraffini et al., 2010; Inek et al., 2012).

3. Applications of CRISPR-Cas in Crop Improvement

CRISPR/Cas9 gene editing has been successfully used to produce crop disease resistance varieties and to improve tolerance level to major environmental stresses. Studies reported by Shan et al., (2013) on rice genes, betaine aldehyde dehydrogenase (OsBADH2), mitogen-activated protein kinase (OsMPK2), phytoene desaturase (OsPDS), which is responsible for abiotic stresses selected for gene editing by CRISPR-Cas9 technique was tested in rice protoplast. A CRISPR/Cas9 mediated genome editing in the ethylene responsive factor, OsERF922 in rice, shows increase resistance against the pathogen *Magnaporthe oryzae* that causes blast disease (Liu et al., 2012). Gene editing is also demonstrated in wheat, CRISPR TaMLO knockout show resistance to to powdery mildew disease caused by *Blumeria graminis* f. sp. *Tritici* (Btg). Kim et al. (2018) studies in wheat protoplasts for wheat dehydration responsive element binding protein 2 (TaDREB2) and wheat ethylene responsive factor 3 (TaERF3), around 70% of protoplasts were successfully expressed. Similarly, the gene involved in the synthesis of antinutritional factors (phytic acid) was targeted knock out of genes ZmIPK1A, ZmIPK, and ZmMRP4 (Liang et al., 2014). Furthermore, carotenoid biosynthesis gene (PSY1) in maize was modified by maize U6 snRNA promoter resulted in white kernels and albino seedlings (Zhu et al., 2016). Studies on Arabidopsis by Feng et al. (2013) showed CRISPR/Cas9 based target genome editing, three phenology related Arabidopsis genes, brassinosteroid insensitive1 (BRI1), jasmonate-zim-domain protein 1 (JAZ1) and gibberellic acid insensitive (GAI) observed in succeeding generations. CRISPR/Cas9 genetic improvement studies on the *Wx* gene in the japonica rice variety successfully produced 5–12% grain amylose content. Knockout of gene *DcMYB7*, a *R2R3-MYB*, in the solid purple carrot resulted in yellow roots (Xu et al., 2019). Most of the World Trade Organization members are supporting the use of gene editing in agricultural innovation; this was the first step towards establishing a worldwide regulatory framework for hunger free world (Liu et al., 2021).

4. Conclusion:

CRISPR/Cas mediated gene editing is a game changing technique with wide application in crop improvement to increase yield, nutritional value, disease resistance and tolerance to environmental stress. In the last decade, it is being used in many plant systems both in dicots and monocots to combat abiotic and biotic stresses and to improve desirable agronomic traits. However, CRISPR/Cas9 based genome editing is gaining popularity with several modifications to obtain suitable edited desired plants that will help achieve the zero hunger sustainable goals to the growing human population.

References:

1. Jaganathan, D., Ramasamy, K., Sellamuthu, G., Jayabalan, S., & Venkataraman, G. (2018). CRISPR for crop improvement: An update review. *Frontiers in plant science*, *9*, 985.
2. Fiaz, S., Ahmar, S., Saeed, S., Riaz, A., Mora-Poblete, F., & Jung, K.H. (2021). Evolution and application of genome editing techniques for achieving food and nutritional security. *International journal of molecular sciences*, *22*(11), 5585.
3. Gaj T., Gersbach C.A. Barbas, C.F., III. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. *Trends Biotechnol.* 2013; 31: 397–405. https://doi.org/10.1016/j.tibtech.2013.04.004.
4. Matres, J.M., Hilscher, J., Datta, A., Armario-Nájera, V., Baysal, C., He, W., ... & Slamet-Loedin, I.H. (2021). Genome editing in cereal crops: An overview. *Transgenic research*, *30*, 461-498.
5. Ansari, W.A., Chandanshive, S.U., Bhatt, V., Nadaf, A.B., Vats, S., Katara, J.L., ... & Deshmukh, R. (2020). Genome editing in cereals: Approaches, applications and challenges. *International Journal of Molecular Sciences*, *21*(11), 4040.
6. Makarova, K.S., Wolf, Y.I., Alkhnbashi, O.S., Costa, F., Shah, S.A., Saunders, S.J., ... & Koonin, E.V. (2015). An updated evolutionary classification of CRISPR–Cas systems. *Nature Reviews Microbiology*, *13*(11), 722-736.
7. Hille, F., & Charpentier, E. (2016). CRISPR-Cas: Biology, mechanisms and relevance. *Philosophical transactions of the royal society B: Biological sciences*, *371*(1707), 20150496.
8. Babu, M., Beloglazova, N., Flick, R., Graham, C., Skarina, T., Nocek, B., ... & Yakunin, A.F. (2011). A dual function of the CRISPR–Cas system in bacterial antivirus immunity and DNA repair. *Molecular microbiology*, *79*(2), 484-502.
9. Wei, Y., Terns, R.M., & Terns, M.P. (2015). Cas9 function and host genome sampling in Type II-A CRISPR–Cas adaptation. *Genes & development*, *29*(4), 356-361.
10. Silas S, Mohr G, Sidote DJ, Markham LM, Sanchez-Amat A, Bhaya D, Lambowitz AM, Fire AZ. 2016 Direct CRISPR spacer acquisition from RNA by a natural reverse transcriptase-Cas1 fusion protein. Science 351, paad4234.
11. Carte, J., Wang, R., Li, H., Terns, R.M., & Terns, M.P. (2008). Cas6 is an endoribonuclease that generates guide RNAs for invader defense in prokaryotes. *Genes & development*, *22*(24), 3489-3496.
12. Deltcheva, E., Chylinski, K., Sharma, C.M., Gonzales, K., Chao, Y., Pirzada, Z.A., ... & Charpentier, E. (2011). CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III. *Nature*, *471*(7340), 602-607.
13. Deveau, H., Barrangou, R., Garneau, J.E., Labonté, J., Fremaux, C., Boyaval, P., ... & Moineau, S. (2008). Phage response to CRISPR-encoded resistance in Streptococcus thermophilus. *Journal of bacteriology*, *190*(4), 1390-1400.
14. Marraffini, L.A., & Sontheimer, E.J. (2010). Self versus non-self discrimination during CRISPR RNA-directed immunity. *Nature*, *463*(7280), 568-571.
15. Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A., & Charpentier, E. (2012). A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. *science*, *337*(6096), 816-821.
16. Wang, B., Xie, G., Liu, Z., He, R., Han, J., Huang, S., ... & Cheng, X. (2019). Mutagenesis reveals that the OsPPa6 gene is required for enhancing the alkaline tolerance in rice. *Frontiers in plant science*, *10*, 759.
17. Xu, Z.S., Yang, Q.Q., Feng, K., & Xiong, A.S. (2019). Changing carrot color: Insertions in DcMYB7 alter the regulation of anthocyanin biosynthesis and modification. *Plant. physiology*, *181*(1), 195-207.
18. International Statement on Agricultural Applications of Precision Biotechnology. [(accessed on 1 April 2021)]; 2018 Available online: https://docs.wto.org/dol2fe/Pages/FE_Search/FE_S_S009-DP.aspx?language=E&CatalogueIdList=250406,249838,249823,249748,249641,249507,249371,249321,249324,249267&CurrentCatalogueIdIndex=7&FullTextHash=&HasEnglishRecord=True&HasFrenchRecord=True&HasSpanishRecord=True.
19. Liu Q, Yang F, Zhang J, Liu H, Rahman S, Islam S, Ma W, She M. Application of CRISPR/Cas9 in Crop Quality Improvement. Int J Mol Sci. 2021 Apr 19;22(8):4206. https://doi.org/10.3390/ijms22084206. PMID: 33921600; PMCID: PMC8073294.