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Application of CRISPR/Cas9 Genome Editing Technology in Modern Agriculture

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Introduction:

Modern advances of molecular genome editing technology like the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/Cas9), a RNA guided endonuclease can be used to modify almost any genomic sequence with known Protospacer Adjacent Motif (PAM) to get a desired phenotype and it has already resolved major challenges in crop improvement like plant disease and insect pest resistance, tolerance to climate changes, yield and quality improvement etc. in nearly 20 different plant species. CRISPR/Cas9 system was initially identified as a microbial adaptive immune system in bacterial cells like *E. coli* to resist bacteriophages (Ishino*et al*., 2018). In CRISPR/Cas9 system, 20 bp long single guide RNA (a tracrRNA-crRNA chimera) is designed as a complementary sequence of the target gene so that it can bind to it and Cas9 is a nuclease that creates single stranded or double stranded breaks followed by the cell's own DNA repair machinery through either Non-Homologous End Joining (NHEJ) or Homologous Directed Repair (HDR) mechanisms in presence of a donor DNA sequence (Cui *et al*., 2018).

CRISPR/Cas9 system provides a huge opportunity to the plant breeders to improve an elite genotype for multiple traits within very short time unlike time consuming back crossing method of conventional breeding (Wade., 2015). Few reports have been discussed herein where this technique was successfully applied.

Yield improvement:

In rice, previously known negative regulators of yield like grain number (OsGn1a), grain size (OsGS3), grain weight (TaGW2, OsGW5, OsGLW2, or TaGASR7), panicle size (OsDEP1, TaDEP1), tiller-spreading (LAZY1), tiller number (OsAAP3), grain weight–related genes (GW2, GW5, and TGW6) were knocked out leading to improved phenotypes (Miao *et al*., 2013, Shan *et al*., 2014, Jiang *et al*., 2013). As yield is a complex quantitative character, modification of a single factor will not be effective at field level, but this CRISPR/Cas9 system has helped breeders in large scale identification of the gene functions.

Quality improvement:

A mutation in the betaine aldehyde dehydrogenase 2 (BADH2) gene leads to biosynthesis of 2-acetyl-1-pyrroline, the major fragrance compound in aromatic rice. With the help of CRISPR/Cas9, this trait has been improved in nearly 30 rice genotypes in China (Chen *et al*., 2019). Low gluten wheat lines have been developed by knocking out the most conserved domains of α-gliadin family members (Sánchez-León., 2018). By DuPont Pioneer waxy maize with improved digestibility has been developed by knocking out the maize waxy gene (Wx1).

Biotic and abiotic stress tolerance:

Taedr1 wheat plants resistant to powdery mildew were developed by simultaneous modification of the three homeologs of EDR1. Blast disease and bacterial blight resistant rice varieties, powdery mildew and bacterial speck resistant tomato varieties were also reported to be developed using CRISPR/Cas9 (Nekrasov*et al*., 2017). Rice varieties with modified OsCYP71A1 gene showed reduced serotonin biosynthesis with increased salicylic acid levels leading to plant hoppers and stem borer resistance. Drought tolerance was also studied in tomato and rice by modifying genes like SlNPR1 and OsPDS respectively (Li *et al*., 2019).

Miscellanies:

Male sterile lines in maize and rice were developed during hybrid seed production by knocking out the gene TMS5. Transgene free genome editing, DNA base editing, Multiplex genome editing have been done successfully in plants like Arabidopsis, tobacco, lettuce, and rice etc. using CRISPR/Cas9 technique (Zhang *et al*., 2018).

Challenges and Future prospects:

CRISPR/Cas9 implementation especially in agriculture faces a great challenge in its effective delivery machinery in accurate plant cells, subsequent regeneration of plants and expression in the viable plants. For high efficiency genome editing in plants, a novel delivery system like direct delivery to plant apical meristems or pollen grains to obtain edited plants is urgently required. Besides public acceptance for modified agricultural commodities is also a key factor. In near future this simple, affordable, and elegant genetic scalpel, CRISPR/Cas9 will gain much more popularity among the breeders to achieve zero hunger goals with suitably edited new plant types.

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