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CRISPR-Cas9: New Breeding Technique

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Introduction:

The CRISPR/Cas9 system is an RNA-guided genome-editing tool. It contains a Cas9 nuclease and a single-guide RNA (sgRNA). Sg-RNA binds with the target DNA sequence and enables the site-directed nucleases activity of Cas9. This process generates double-strand breaks (DSBs) that trigger cell repair mechanisms. This repair mechanism is error-prone and develops mutations(nonworking genes). CRISPR is being widely utilized to edit thegenomes of a diverse range of crop plants.

CRISPR-Cas9 offers the opportunity to quickly introduce new traits into crops and livestock whilst bypassing traditional breeding methods. These traditional selective breeding methods suffer from low efficacy and are limited by the alleles present in the population. Improved techniques, such as marker-assisted selection(MAS), are based on a more recent understanding of genetics and cellular biology though still lack the accuracy and precision that can be achieved using CRISPR-Cas9 and other NBTs. NBTs(New Breeding Technique) including CRISPR-Cas9 alongside Zinc-Finger Nucleases (ZFNs) and Transcription Activator-Like Effector Nucleases (TALENs).

APPLICATIONS

1.1. Increasing yields.

Yield is a complex trait that is quantitative and controlled by quantitative trait loci. Knocking out negative regulators known to affect yield-determining factors such as grain number, grain size, grain weight, panicle size, and tiller number created the expected phenotypes in plants with loss-of-function in these genes, demonstrating that CRISPR/Cas9 is an effective tool for improving yield-related traits.

1.2. Improving quality.

Quality traits vary depending on the specific plants. Rice with low amylose content was generated by knockout of *Waxy* via CRISPR/Cas9. The betaine aldehyde dehydrogenase 2 (*BADH2*) gene can be deleted to increase the biosynthesis of 2-acetyl-1-pyrroline, the major fragrance compound in fragrant rice. Anti-browning mushroom created by deleting a polyphenol oxidase (PPO) gene will become the first organism edited using the CRISPR system to be given the go-ahead by the US government.

Other high-quality crops produced by CRISPR/Cas9 editing include seeds with high oleic acid oil in *Camelina sativa* and *Brassica napus*, low-gluten wheat, tomatoes with a long shelf life, high-value tomato with enhanced lycopene or γ-aminobutyric acid content, and potato (hairy roots) with reduced levels of toxic steroidal glycoalkaloids.

1.3. Biotic- and abiotic-stress resistance.

Biotic- and abiotic-stress are the main factors affecting crop yield and quality. With the help of CRISPR/Cas9 knockout many plants with increased biotic-stress resistance, including resistance to fungal, bacterial, and viral diseases and insects, have been obtained. TALEN and CRISPR/Cas9 were used to knock out all six *TaMLO* alleles in wheat and obtained plants with increased resistance to powdery mildew. CRISPR/Cas9-mediated knockout of *MLO* rise resistance to powdery mildew in tomato. Blast-resistant rice was obtained via knockout of *OsERF922*. Disrupting *OsCYP71A1* blocked serotonin biosynthesis and greatly increased salicylic acid levels, thereby conferring resistance to planthoppers and stem borers, the two most destructive pests of rice. *pyl1/4/6* triple knockout in rice created by CRISPR/Cas9 editing had increased grain yield, greater high-temperature tolerance, and reduced preharvest sprouting compared with wild type.

1.4. Speeding hybrid breeding.

A male-sterile maternal line is essential for producing a high-quality hybrid variety. Many male-sterile lines have been produced by CRISPR/Cas9 mediated gene knockout technology, such as thermosensitive male-sterile *tms5* lines in rice and maize, photosensitive genic male-sterile *csa* rice, and *ms45* wheat. To tame hybrid sterility in *japonica-indica* hybrids, *SaF/SaM* at the sterility locus *Sa* and *OgTPR1* at the *S1* locus were disrupted.

Genome editing is also an effective approach for enhancing many other traits, such as improving haploid breeding, shortening growth times, increasing silique shatter resistance, overcoming self-incompatibility in diploid potato, and develop asexual propagation lines to meet breeders' requirements.

Conclusions

CRISPR-Cas9 is a revolutionary tool that can impact science, food production, and society. CRISPR-Cas9 has great potential for transforming agriculture by improving their nutritional value, yield and making plants tolerant to biotic- and a biotic stresses. These attributes make CRISPR-Cas9 an important tool to meet the demand of an increasing world population.