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Precision Breeding: How CRISPR Is Shaping the Next Generation of Wheat

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Common wheat (*Triticum aestivum* L.) is a major global food crop whose productivity is increasingly threatened by climate change, biotic and abiotic stresses, and limited arable land. Genetic improvement in wheat is challenging due to its large and complex hexaploid genome, which restricts the efficiency of conventional breeding approaches. Recent advances in CRISPR/Cas genome editing technologies have provided powerful and precise tools for targeted genetic manipulation in wheat. This article highlights the application of CRISPR/Cas-based systems, including CRISPR/Cas9-mediated mutagenesis, base editing, prime editing, multiplex editing, and transgene-free approaches, for wheat improvement. These technologies have been successfully used to enhance grain yield, nitrogen use efficiency, disease resistance, grain quality, nutritional value, and tolerance to abiotic stresses. Notably, simultaneous editing of multiple homeologous gene copies has enabled effective trait improvement despite genome redundancy. Although challenges such as transformation inefficiency, off-target effects, regulatory uncertainty, and validation complexity persist, continuous technological advancements are improving editing efficiency and precision. Overall, CRISPR/Cas genome editing represents a transformative approach for accelerating wheat breeding and developing high-yielding, climate-resilient, and nutritionally enhanced wheat varieties, thereby contributing significantly to sustainable agriculture and global food security.

Keywords: Common wheat, CRISPR/Cas genome editing; wheat improvement; hexaploid genome; multiplex genome editing; disease resistance; abiotic stress tolerance; grain quality; transgene-free editing; climate-resilient crops; sustainable wheat breeding

Introduction

Common wheat (*Triticum aestivum*) is one of the most widely cultivated and consumed crops globally. In the face of limited arable land and climate changes, it is a great challenge to maintain current and increase future wheat production. Enhancing agronomic traits in wheat by introducing mutations across all three homoeologous copies of each gene has proven to be a difficult task due to its large genome with high repetition. However, clustered regularly short palindromic repeat (CRISPR)/CRISPR-associated nuclease (Cas) genome editing technologies offer a powerful means of precisely manipulating the genomes of crop species, thereby opening up new possibilities for biotechnology and breeding. Recently developed and optimized CRISPR/Cas-mediated genome editing systems including targeted gene mutagenesis, base editing, prime editing (PE), and multiplex genome editing, are now being applied to common wheat, providing genetic tools with unparalleled precision and efficiency for advancing wheat breeding.

Genome editing tools used in wheat

- **CRISPR/Cas9:** The most widely used genome editing system due to its precision, efficiency, and multiplexing capacity. Variants such as cytidine base editors, adenosine base editors, and prime editors have also been adapted for wheat to create precise point mutations.
- **Transgene-free editing:** Techniques such as delivering CRISPR/Cas9 as ribonucleoproteins (RNPs) enable the production of non-GMO, transgene-free edited plants, which is particularly advantageous for regulatory approval and market acceptance.

CRISPR/Cas9 genome editing in wheat

The CRISPR/Cas9 system, a Class 2 Type II CRISPR system, consists of the Cas 9 nuclease and a single guide RNA (sgRNA). The CRISPR/SpCas9 system from *Streptococcus pyogenes*, recognizing the 5′-NGG-3′ protospacer adjacent motif (PAM), was the first to achieve specific DNA cleavage in plant cells.

Step-by-step mechanism

- **Recognition:** A single guide RNA (sgRNA), designed to match the target wheat gene sequence, forms a complex with the Cas9 protein. The sgRNA directs Cas9 to the specific DNA region by complementary base pairing.
- **Cleavage:** Cas9, guided by the sgRNA, scans the wheat genome and binds to the DNA only at the presence of a specific adjacent motif called the PAM (protospacer adjacent motif), typically 'NGG' for Streptococcus pyogenes Cas9. The Cas9 endonuclease introduces a double-strand break (DSB) three base pairs upstream of the PAM sequence. The HNH domain of Cas9 cuts the DNA strand complementary to the sgRNA, while the RuvC domain cuts the opposite strand, generating blunt-ended DSBs.
- **Repair:** The cell repairs these DSBs primarily via the non-homologous end joining (NHEJ) pathway, which is error-prone and often results in insertions or deletions (indels) at the target site. This can lead to gene knockout or disruption. Alternatively, homology-directed repair (HDR) can be used if a repair template is provided, allowing precise insertion or alteration in the genome.

Key target traits improved by genome editing

CRISPR genome editing has enabled precise improvement of several key traits in wheat, addressing yield, quality, disease resistance, nutritional value, and stress tolerance. The major traits improved through this technology are:

1. Grain yield and agronomic traits

Genome editing is harnessed to improve yield-related characters:

- **Grain size and weight:** Editing auxin response factor *TaARF12* produced plants with shorter stems and larger spikes, resulting in up to 14.7% more grains per spike and up to 11.1% yield increase in field trials.
- **Manipulation of regulatory pathways:** Editing genes like those in the CLAVATA-WUSCHEL pathway (controlling meristem size) and genes involved in nitrogen use efficiency (e.g., ARE1 ortholog) have produced wheat varieties with increased grain yield under various conditions.
- **Delayed senescence:** Knockout of TaARE1 improves nitrogen use efficiency and delays plant aging, further boosting grain yield.
- **Root architecture:** Editing genes such as *TaRPK1* can modify root system architecture to enhance nutrient uptake and yield.
- **Rapid pyramiding of favourable alleles:** Multiplex gene editing allows for simultaneous improvement of several agronomic traits in a single generation.

2. **Disease resistance**

Plant diseases like rust, fusarium head blight, and powdery mildew significantly impact wheat productivity. Genome editing strategies include:

- **Introduction of resistance (R) genes:** Targeting and inserting novel or enhanced R genes for durable resistance.
- **Modification of susceptibility (S) genes:** Knocking out S genes (such as the *Mlo* gene) to confer broad-spectrum resistance, particularly to powdery mildew.
  - ✓ Successful editing of *Mlo* resulted in wheat varieties resistant to powdery mildew without negative pleiotropic effects.
  - ✓ The knockout of all *TaMPK1* homeologues increased powdery mildew resistance. CRISPR/Cas9-mediated knockout of *TaMPK1* not only improved resistance to powdery mildew but also enhanced stripe rust resistance, another biotrophic pathogen of wheat.
  - ✓ Similarly, the CRISPR/Cas9-mediated knockout of *TaGW2* and *TaWRKY19* also conferred resistance to leaf and stripe rust in wheat, respectively.
  - ✓ The CRISPR/Cas9 knockout of *TaHRC-3B* improved FHB resistance, offering a faster alternative to backcrossing for introducing this locus into wheat varieties.
  - ✓ Similarly, the knockout of all six *TaNFXL1* gene copies, a gene associated with deoxynivalenol toxin sensitivity, also enhanced FHB resistance.
  - ✓ CRISPR/Cas9-mediated knockout of *TaeIF4E* homeologues increased resistance to wheat yellow mosaic virus without yield penalty.
- **Engineering immune receptors:** Manipulation of nucleotide-binding leucine-rich repeat receptors (NLRs) to expand disease resistance spectrum.

3. **Grain quality**

Grain quality is governed by several genes involved in starch and protein synthesis, colour, and processing qualities:

- **Hardness, starch composition, and dough colour:** Genes such as *pinb*, *waxy*, *ppo*, and *psy* have been edited for desirable quality traits.
  - ✓ **pinb**: Involved in regulating grain hardness, which affects milling and baking quality.
  - ✓ **waxy**: Controls starch composition, influencing amylose content and thus the texture and processing properties of wheat-based foods.
  - ✓ **ppo**: Polyphenol oxidase gene, related to the dough colour—decreasing its activity leads to whiter flour and better appearance.
  - ✓ **psy**: Phytoene synthase gene, involved in grain colour and the synthesis of carotenoids, impacting nutritional quality and visual appeal.
- **Gluten strength and protein content:** Editing specific quality-associated genes has yielded wheat with improved processing and nutritional qualities, including enhanced protein and gluten strength.
- **Nutritional enhancement:** Targeted editing has improved accumulation of micronutrients (e.g., iron, zinc), vitamins (e.g., provitamin A, vitamin E), and reduced anti-nutritional factors like phytic acid by modifying the TaABCC6 gene.

4. **Abiotic stress tolerance**

- **Stress-responsive genes:** Editing transcription factors such as *TaDREB2* (dehydration responsive element binding protein), and *TaERF3* (ethylene response factor) increases resistance to heat, drought, and salinity. Manipulating genes that control root development can improve water and nutrient uptake, increasing yield stability on marginal soils and under water-limiting conditions.

5. **Hybrid seed production and trait pyramiding**

- CRISPR/Cas9 allows efficient multi-gene editing to combine several beneficial traits rapidly, expediting breeding cycles and trait stacking efforts in wheat.

6. **Stability and multiplexing**

- CRISPR enables multiplex genome editing (simultaneous alteration of multiple genes or alleles in wheat's complex hexaploid genome), offering a major advantage over classical breeding approaches.

Advantages and innovations

- **Speed and precision:** Genome editing provides faster results than traditional breeding and mutagenesis, allowing targeted improvement of specific traits without the need for multiple plant generations.
- **Multiplexing:** Multiple genes can be edited simultaneously, enabling complex trait improvement in a single recombination event.
- **Transgene-free plants:** DNA-free approaches ensure edited wheat varieties are non-GMO, easing regulatory hurdles.
- **Heritable and stable edits:** Edits are inheritable, with trait stability maintained over subsequent generations.

Challenges in applying CRISPR technology for wheat breeding

1. Polyploidy complexity

- **Hexaploid genome:** Wheat has a highly complex hexaploid genome (~17 billion base pairs) with three closely related sub-genomes. This demands precise targeting of all gene copies (homeologs) for visible phenotypic changes.
- **Gene redundancy:** The presence of multiple gene copies often requires simultaneous editing of several homeologs, complicating both guide RNA design and validation of edits.

2. Transformation and regeneration barriers

- **Low transformation efficiency:** Wheat remains a recalcitrant species for genetic transformation, with varietal differences in amenability to tissue culture and regeneration.
- **Genotype dependence:** Transformation methods commonly work in a limited number of elite or model varieties, restricting broad applicability across diverse wheat germplasm.
- **Labor-intensive protocols:** Tissue culture and plant regeneration procedures are time-consuming and technically demanding.

3. Off-target effects

- **Precision needs:** While CRISPR/Cas9 is highly targeted, off-target mutations can still occur, potentially affecting non-target genes and resulting in unintended phenotypes.
- **Complex genome increases risk:** The repetitive and large wheat genome heightens the risk of off-target effects, making rigorous screening essential.

4. Regulatory and public acceptance

- **Ambiguous regulatory landscape:** Global regulatory frameworks for genome-edited crops are evolving. In some countries, minor DNA edits in plants may be regulated similarly to GMOs, complicating commercialization.
- **Consumer concerns:** Uncertainties surrounding gene-edited foods can hinder acceptance and adoption, even if the end-product is transgene-free.

5. Multiplex editing difficulties

- **Technical constraints:** Achieving efficient, simultaneous editing of multiple genes or gene copies is challenging, but necessary for traits governed by polygenic control or redundancy.
- **Guide RNA optimization:** Designing and delivering effective multiplex guide RNAs without loss of efficiency remains a technical hurdle.

6. Validation and screening

- **High throughput genotyping required:** Detecting and confirming edits, especially homozygous or transgene-free edits, in polyploid wheat requires resource-intensive screening of many plants over multiple generations.
- **Phenotyping complexity:** Linking genotype to phenotype, particularly for traits with environmental interactions, adds to the complexity.

7. Delivery of editing machinery

- **DNA-free editing limitations:** Although ribonucleoprotein (RNP)-based delivery can avoid transgene integration, this approach currently has lower efficiency and is less developed in wheat compared to DNA-based methods.

8. Intellectual property and access

- **Patent and licensing issues:** Widespread patents on CRISPR components can limit access to necessary tools, especially for public-sector breeding programs.

Conclusion

In the coming years, worldwide wheat production will significantly reduce due to climate change, poor irrigation, and excessive use of chemical fertilizers, leading to poor soil quality. To tackle this problem, we must develop a strong breeding pipeline to identify genes and molecular signatures to characterize, manipulate, and validate the major traits of wheat like abiotic stress tolerance, disease resistance, agronomic/breeding traits and grain quality. Traditional wheat breeding methods face limitations like high costs, lengthy timelines, and potential loss of desired traits over generations. However, the CRISPR-Cas9 genome editing tool offers a precise and efficient approach for targeted genetic modifications in wheat. Its integration into breeding programs promises acceleration of superior wheat cultivars with improved quality, productivity, and stress tolerance. As this technology continues advancing, it holds immense potential in addressing food security challenges, enhancing crop productivity, and developing climate-resilient wheat varieties, paving the way for a more sustainable food for future.

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