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REVIEW

CRISPR/Cas9 genome editing in wheat: enhancing quality and productivity for global food security—a review

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Abstract

Wheat (*Triticum aestivum* L.) is an important cereal crop that is grown all over the world for food and industrial purposes. Wheat is essential to the human diet due to its rich content of necessary amino acids, minerals, vitamins, and calories. Various wheat breeding techniques have been utilized to improve its quality, productivity, and resistance to biotic and abiotic stress impairing production. However, these techniques are expensive, demanding, and time-consuming. Additionally, these techniques need multiple generations to provide the desired results, and the improved traits could be lost over time. To overcome these challenges, researchers have developed various genome editing tools to improve the quality and quantity of cereal crops, including wheat. Genome editing technologies evolve quickly. Nowadays, single or multiple mutations can be enabled and targeted at specific loci in the plant genome, allowing controlled removal of undesirable features or insertion of advantageous ones. Clustered, regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein (Cas) is a powerful genome editing tool that can be effectively used for precise genome editing of wheat and other crops. This review aims to provide a comprehensive understanding of this technology's potential applications to enhance wheat's quality and productivity. It will first explore the function of CRISPR/Cas9 in preserving the adaptive immunity of prokaryotic organisms, followed by a discussion of its current applications in wheat breeding.

Keywords Genome editing · CRISPR/Cas9 · Abiotic stress · Biotic stress · Wheat · *Triticum aestivum* L

Introduction

Wheat is the most essential crop used to provide staple foods for more than 33% of the world's population (Grote et al. 2021). It is the most widely cultivated crop in the world, cultivated on 217 million ha annually (Erenstein et al. 2022), and it contributes more than 20% of the total calories humans consume (Gupta et al. 2021). Production of wheat in 2022 was estimated to be 781 million tons. China is the largest wheat producer, with 138 million tons (USDA 2022). Wheat is an allohexaploid ($2n = 6 \times = 42$, AABBDD) with three closely related subgenomes inherited from three homoeologous ancestors. As a result, most wheat genes have three similar but not identical copies, leading to functional redundancy and complementarity among the A, B, and D genomes (Li et al. 2021c). Climate change has increased severe weather events, including heat, drought, and heavy metals (Iordache et al. 2022). The abiotic stress caused a global wheat production loss of 11.1 million tons in 2022. The most damaging factor was drought, which accounted for 44% of the total losses (FAO 2022a). Climate change is expected to reduce worldwide wheat output by 1.9% by mid-century, with the impact being seen most strongly in Africa and Southern Asia, where yield declines of 15% and 16% are forecast by 2050 (Pequeno et al. 2021). Globally, temperature increases lowered wheat productivity by 6% per degree Celsius (Basile et al., 2022). Biotic factors, including fungi, bacteria, viruses, and pests, pose are responsible for considerable losses, ranging between 20 and 40% of global agricultural productivity (Boubakri 2023). The biotic stresses caused wheat production losses

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of 12.5 million tons (FAO 2022b). The most severe biotic stress during that year was rust, which accounted for 60% of the total losses (Bhavani et al. 2022) (Fig. 1). If future agricultural output is not high enough to feed the expanding population of the globe, then there is a strong possibility that there will be unprecedented levels of human suffering (Levy et al. 2017).

To combat the growing abiotic and biotic stresses impacting agricultural production, both publicly sponsored institutions and commercial firms need to expedite cultivar development (Qaim 2020). The utilization of genomic data can enhance traditional breeding methods through approaches such as marker-assisted selection (MAS), genomic selection (GS), and genome-wide association studies (GWAS), enabling more accurate and faster selection of desired traits (Zhang et al. 2016). Furthermore, recent techniques, including CRISPR/Cas9 genome editing, can be employed to create precise genetic changes for improved disease resistance or environmental adaptability (Son and Park 2022). Nevertheless, the ethical and regulatory complexities surrounding genetically modified organisms (GMOs) necessitate robust public engagement and appropriate policy-making. By complementing these technological solutions with sustainable farming practices, it is possible to address global agricultural challenges more holistically (Catacora-Vargas et al. 2018). In 2012, scientists discovered that a bacterial immune system endonuclease could be tailored to particular DNA sequences (Adli 2018). This method, first utilized in plants in 2013, has recently been used in developing commercial plant products by incorporating targeted DNA mutations (Nadakuduti and Enciso-Rodríguez 2021).

This review focuses on the potential of CRISPR/Cas9 in wheat breeding improvement. Genetic modification of the intricate wheat genome has posed challenges in understanding and modifying its genes. The review introduces the underlying mechanisms of CRISPR/Cas9 in prokaryotic adaptive immunity and explores its capacity for precise genomic modifications in wheat plants. Furthermore, it delves into the current applications of CRISPR/Cas9 in wheat breeding and thoroughly discusses this technology's potential and significance in enhancing wheat quality and productivity. The aim is to meet the growing demand for wheat due to the increasing global population and to mitigate the impact of climate change on agriculture.

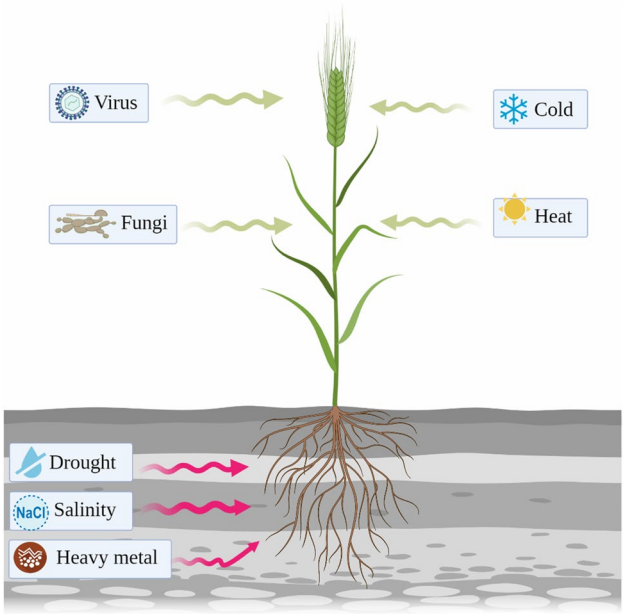

Fig. 1 Illustration showcasing the major abiotic and biotic stresses impacting wheat production. The figure provides an overview of the key stressors, including extreme temperatures, drought, pests, diseases, and soil nutrient deficiencies, which pose significant threats to wheat crops worldwide. The visual representation highlights the interplay between these stress factors and their potential impact on agricultural productivity, emphasizing the need for comprehensive strategies to safeguard wheat cultivation

CRISPR/Cas9 system

Genome editing refers to the insertion, deletion, and replacement of DNA at a particular target region in the genomes of many crops to achieve a range of aims, such as the silencing of genes, the development of new features, or the elimination of harmful mutations (Xu and Li 2020). There are now three common genome editing tools: zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and RNA-guided CRISPR-Cas (Sufyan et al. 2023). CRISPR/Cas9 systems are extensively employed in molecular biology labs throughout the globe because of their simple design, cheap cost, high efficiency, strong reproducibility, and quick cycle (Wang et al. 2020). CRISPR/Cas9 is an adaptive immune system found in most bacteria and archaea, protecting them against phages, viruses, and other foreign genetic material (Li et al. 2021a). CRISPR/Cas9 is found in 45% of bacterial genomes and 83% of archaea (Barman et al. 2020). When prokaryotes are invaded by foreign genetic material, Cas proteins break the DNA into short segments inserted into the CRISPR/Cas9 array as spacers (Gupta et al. 2019). When the same invader attacks again, crRNA will detect it immediately and pair with the foreign DNA, which directs the Cas protein to break specific foreign DNA target regions, defending the host (Xu and Li 2020). The CRISPR/Cas9 technique was initially discovered in 1987 by Ishino, but it largely went unnoticed until a distinctive and peculiar region was unveiled within a draft of a bacterial genome. This discovery renewed attention to the CRISPR system and sparked further investigations into its potential functions and applications (Gostimskaya 2022).

Genome editing using CRISPR/Cas9 systems

The Cas9 endonuclease and sgRNA can target practically any genomic location and cause double-stranded breaks (DSBs) (Manghwar et al. 2020). DSBs are repaired by either the imprecise non-homologous end-joining (NHEJ) repair pathway or the precise homology-directed repair (HDR) pathway (Tang et al. 2019). NHEJ can yield gene knockouts, and HDR can modify DNA sequences (Eid et al. 2018). In higher plants, NHEJ occurs most frequently than the more precise HDR. HDR requires a donor DNA template during homologous recombination to repair the dsDNA breaks (Molla et al. 2021) (Fig. 2). This has several opportunities for designing single-base alterations, diversifying a localized sequence, developing new protein variants, and speeding the evolution of certain proteins to create agricultural cultivars that can withstand biotic or abiotic challenges (Shimatani et al. 2017).

CRISPR/Cas9 for enhancing crop quality and productivity

Plants are exposed to different environmental stresses, encompassing biotic stresses caused by microbes and abiotic stresses resulting from climatic changes. The combined effect of these challenges contributes to nearly 50% of global crop yield losses (Alqudah et al. 2020). Crop breeding has significantly influenced food supply across agriculture's development thousands of years ago (Tabassum et al. 2021).Through selective breeding, humans have been able to enhance the desired traits of crops, such as yield, disease resistance, and nutritional content. This has led to the cultivation of various crop varieties that can thrive in different climates and environments, increasing food production and contributing to food security (Ahmad et al. 2021). Previously, crop improvement relied on conventional breeding methods, which were time-consuming and labor-intensive (Chaudhry et al. 2023). In recent times, traditional approaches have been complemented and enhanced by modern molecular and genomic-based breeding techniques (Riaz et al. 2021). CRISPR/Cas9 is the latest breakthrough in genome engineering and has profoundly transformed crop breeding since its emergence. By utilizing CRISPR/Cas9, genome editing has become a relatively simple, low-cost, and robust process, resulting in huge advances in crop improvement (Riaz et al. 2022). The CRISPR/Cas9 system has been extensively utilized to improve yield, quality, herbicide resistance, and biotic and abiotic stress tolerance in several plant and crop species (Hussain and Ahmad 2022). The CRISPR/Cas9 system is an efficient tool for targeted genome editing in wheat, showing promise for manipulating the wheat genome to improve crop performance (Kim et al. 2018) (Fig. 3). The applications of CRISPR/Cas9 in wheat genetic manipulation hold great possibilities for enhancing various aspects of wheat

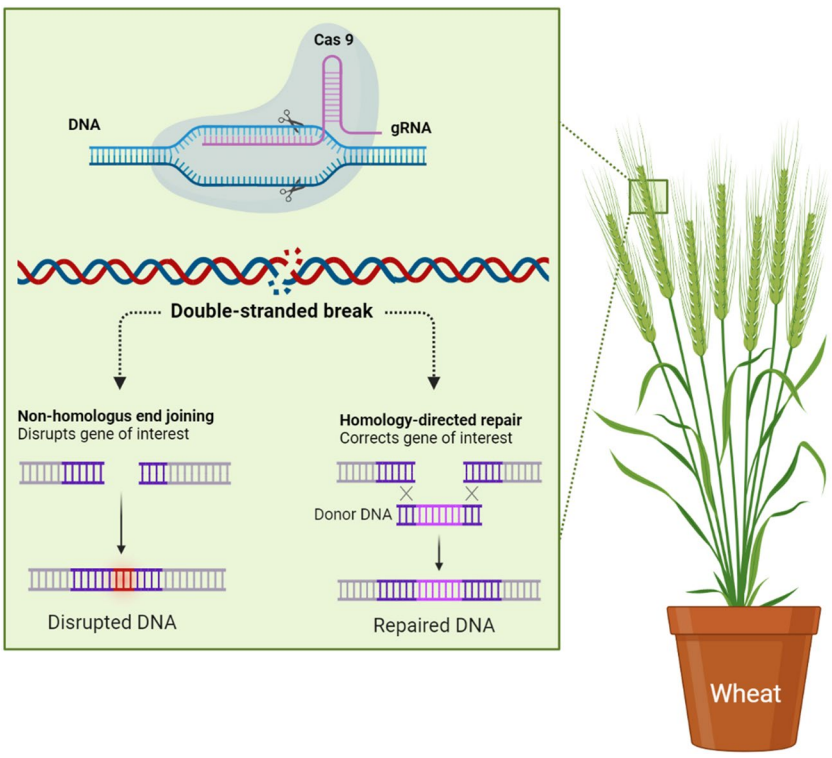

Fig. 2. The application of the CRISPR-Cas9 system in wheat genome editing. The process involves Cas9 nuclease guided by CRISPR RNA (crRNA) and trans-activating CRISPR RNA (tracrRNA) to target and cleave specific DNA sequences in the wheat genome. Subsequent repair mechanisms, such as non-homologous end joining (NHEJ) or homology-directed repair (HDR), lead to the introduction of desired genetic modifications. The CRISPR-Cas9 system offers precise and efficient genome editing capabilities, holding significant promise for crop improvement and agricultural biotechnology

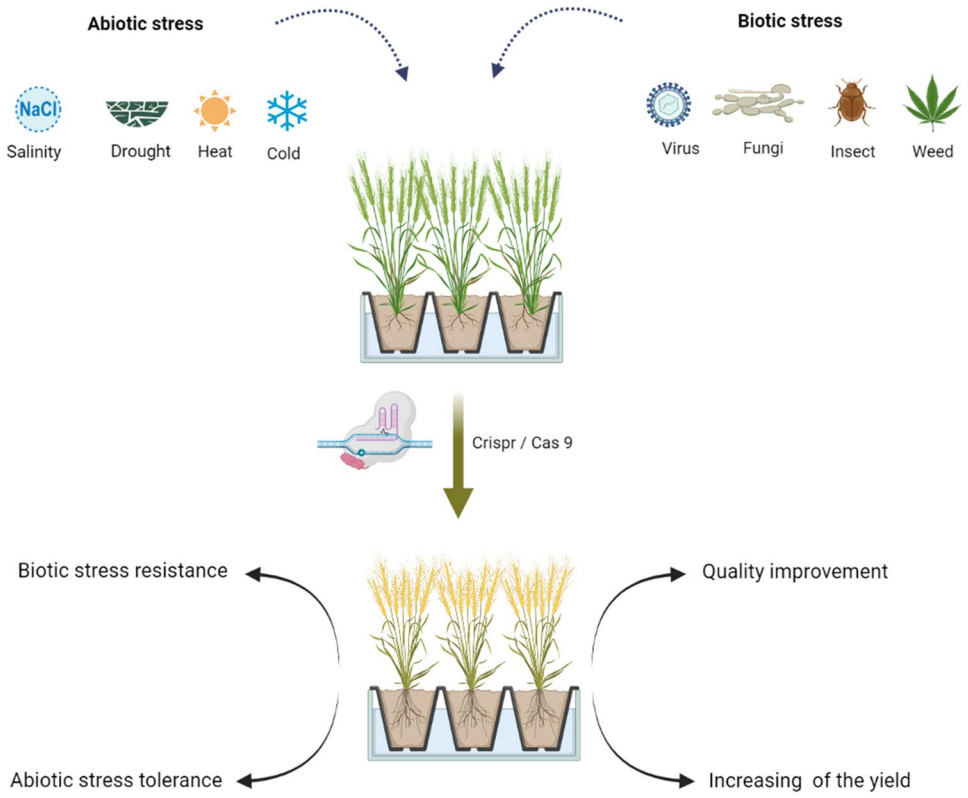

Fig. 3 The use of CRISPR/Cas9 technology to enhance wheat productivity and stress resilience. Researchers applied precise gene editing techniques to modify specific genes in wheat plants, aiming to improve traits related to productivity and stress response, such as drought tolerance and disease resistance

crops, leading to improved crop performance and addressing agricultural challenges (Liu et al. 2021).

CRISPR/Cas9 for enhancing yield quality of wheat

CRISPR/Cas9 technology can be used to improve the quality of wheat by improving different agricultural traits (Fig. 4). Hardness is wheat's distinguishing characteristic. The *puroindoline b gene* (*Pinb*) is a single-copy gene on chromosome 5DS whose absence or alteration by mutation could result in hard texture (Calderini et al. 2021). Standard classifications for wheat include soft, medium-soft, hard, and extra-hard varieties. Grain hardness grades determine the global wheat value (Muqaddasi et al. 2020). Softer wheat kernels may be broken easily, resulting in a high percentage of unbroken starch granules, whereas tougher ones need more energy to mill, yielding a higher percentage of broken starch granules (Muqaddasi et al. 2020). CRISPR/Cas9 technology improved wheat grain quality regarding hardness (Zhang et al. 2021a).

The endosperm of a wheat grain mainly consists of starch, which may be used as a source of energy. The grain starch content made up of the polymers amylose and amylopectin in a ratio of 1:3 impacts the end-use value of bread in various ways, such as dough rheology, bread staling, and crumb structure (Gray and Bemiller 2003). There is a clear link between grain starch components and the quality of wheat flour. Waxy is a major enzyme in wheat endosperm amylose production, encoded by *WxA1*, *WxB1*, and *WxD1* on 7A, 4A, and 7D chromosomes, respectively (Maryami et al. 2020). CRISPR/Cas9 was used to edit the waxy gene in wheat to make it whiter and opaquer, with lower amylose content (Zhang et al. 2021c). Foods high in amylose and resistant starch may promote health and lessen the risk of noninfectious disorders (Wang et al. 2019). Resistant starch refers to any starch or starch derivatives not digested and absorbed in the stomach or small intestine, resulting in lowered blood sugar after human ingestion (Raigond et al. 2015). CRISPR/Cas9-targeted mutagenesis of *TaSBEIIa* generated high amylose wheat with improved resistant starch (Li et al. 2021b). Gluten is the primary protein of wheat grains (Biesiekierski 2017). Gluten proteins contribute to dough's water absorption capacity, cohesivity, viscosity, and elasticity. Gluten proteins are separated into soluble gliadins and insoluble glutenins based on alcohol solubility. Both fractions include similar protein components rich in glutamine and proline (Wieser et al. 2023). Gluten proteins (gliadins and glutenins) in wheat can cause coeliac disease in people genetically more likely to get it (Jouanin et al. 2020). Coeliac disease is an autoimmune response that affects 1–2% of the world's population and is the most frequent illness linked to wheat in humans (Pinto-Sanchez et al. 2021). CRISPR/Cas9

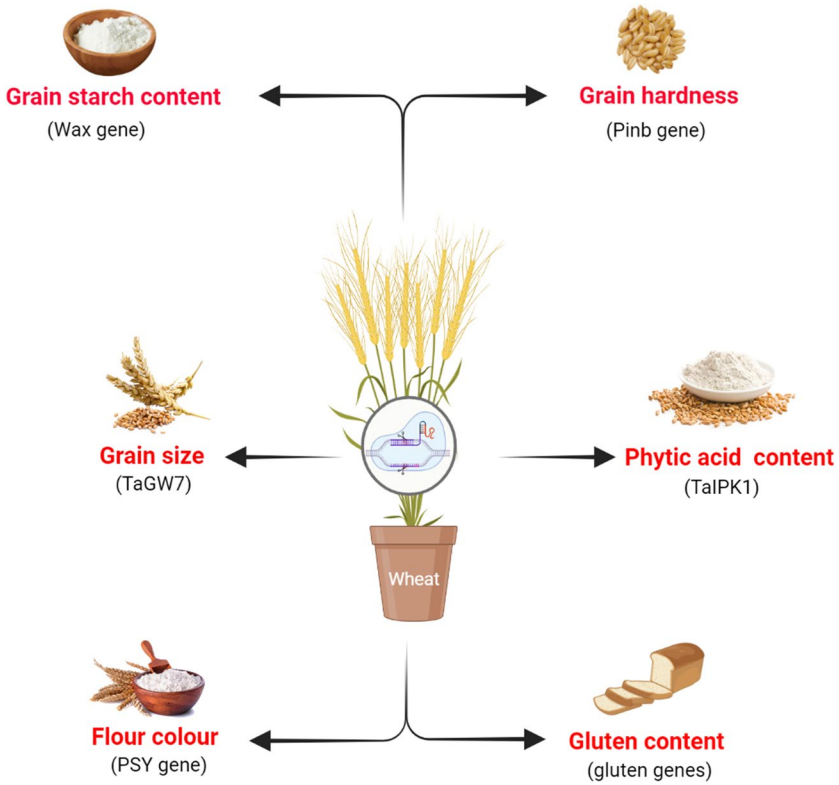

Fig. 4 The CRISPR/Cas9 system is utilized to improve wheat grain quality by precisely editing specific genes related to hardness, gran starch content, grain size, phytic acid content, flour color, and gluten content. This gene editing technology allows for the creation of wheat varieties with softer texture, higher starch content, larger grains, reduced phytic acid, appealing flour color, and modified gluten

is used to develop wheat lines with gluten genes with inactivated coeliac disease epitopes (Jouanin et al. 2020). This has resulted in much less gluten content in wheat (García-Molina et al. 2019).

Polyphenol oxidase (PPO) activity and the amount of yellow pigment in wheat are two important qualities affecting the color of wheat products (Li et al. 2015). PPO is a catalyst that turns phenols into dark-colored compounds, a characteristic often undesired for wheat end-use goods. As a result, one of the primary objectives of wheat breeding has always been to generate cultivars of wheat with low PPO activity (González et al. 2020). CRISPR/Cas9 was used to edit the ppo-7 in wheat to lower PPO activity (Zhang et al. 2021b). Phytoene synthase (PSY) is the most significant regulating enzyme in carotenoid production. The presence of yellow pigment is an essential characteristic for assessing the overall quality of wheat. Increasing the yellow pigment concentration in wheat cultivars in Japan and Southeast Asia is advantageous for making yellow alkaline noodles (Khalid et al. 2019). However, in China, white noodles and steamed bread are all appreciated when they have a brilliant white to creamy color. Therefore, wheat grains with a low yellow pigment are favored (Siah and Quail 2018). As a result, it is crucial for wheat breeding to create new varieties with either a high or low yellow pigment content, depending on the final products that will be made from the grain (Mastrangelo and Cattivelli 2021). CRISPR/Cas9-edited PSY homeologs (*TaPSY-7A*, 7B, and 7D) and *PSY* editing decreased downstream metabolites in the carotenoid biosynthesis pathway (Zhang et al. 2021c). Grain size and weight are essential parts of a set of traits in crops that have to do with production. Wheat grain shape and weight are affected by altering the *TONNEAU1*-recruiting motif encoding gene (Wang et al. 2019). The knockout of *TaGW7* in wheat has been shown to increase grain width and weight (Wang et al. 2019).

Nitrogen fertilizer is widely used to increase wheat yield to meet food demand. Unnecessary nitrogen fertilizer use and low nitrogen use efficiency of modern wheat varieties worsen environmental pollution, and ecological deterioration *TaARE1* gene editing improved wheat's nitrogen use efficiency and increased yield (J. Zhang et al. 2021). Genetic manipulation of spike inflorescence growth might increase grain production (Wolde et al. 2019). The *DUO-B1* regulates spike inflorescence morphology in bread wheat by encoding *APETALA2/ERF* (Wang et al. 2022). *DUO-B1* mutations cause minor supernumerary spikelets, increased grain number per spike, and enhanced yield without changing other agronomic features (Wang et al. 2022). As a negative regulator, *TaIAA21* controls wheat grain size and weight (Jia et al. 2021). The *TaIAA21* mutation improves wheat grain size and weight, improving production (Jia et al. 2021). *TaQ* alleles, a transcription factor in common wheat, influence spike evolution (Liu et al. 2020). The editing of wheat *TaQ* genes using CRISPR/Cas9 results in spike morphogenesis

and grain treatability changes. In addition, it also impacts plant height, flowering duration, and floret structure (Liu et al. 2020).

The accumulation of free asparagine in grains, tubers, beans, storage roots, and other crop products has been studied extensively in recent research, due to its role as a precursor for acrylamide formation during cooking and processing (Raffan et al. 2021). Acrylamide is a processing toxin formed during cooking and processing from free asparagine and reducing sugars (Maan et al. 2022). It is often found in fried, baked, roasted, and toasted meals, such as bread, biscuits, cakes, pies, batter, and morning cereals (Raffan and Halford 2019). According to the International Agency for Research on Cancer, acrylamide is a group 2a carcinogen (Hogervorst and Schouten 2022). Free asparagine content determines acrylamide production in wheat and grain products (Raffan and Halford 2019). There are five different asparagine synthetase genes in each wheat genome. These genes are labelled *TaASN1*, *TaASN2*, *TaASN3*.1, *TaASN3*.2, and *TaASN4*. However, certain wheat types miss a *TaASN2* gene on the B genome (Raffan and Halford 2021). The asparagine synthetase gene *TaASN2* was modified using CRISPR/Cas9 to decrease the accumulation of free asparagine in the grain (Raffan et al. 2021). Low asparagine commercial wheat varieties could be developed, facilitating the production of bread, biscuits, breakfast cereals, and other wheat-based foods with lower levels of acrylamide (Raffan et al. 2021).

Phytic acid is a primary phosphorus (P) source in wheat and other cereals, but monogastric animals, including humans, cannot efficiently use it because they lack phytase enzymes (Sun et al. 2022). Phytic acid lowers iron and zinc in the body, producing malnutrition (Aggarwal et al. 2018). *Inositol pentakisphosphate 2-kinase 1* (*IPK1*) is a phytic acid biosynthesis gene (Pandey et al. 2021). CRISPR/Cas9-mediated disruption of inositol pentakisphosphate 2-kinase 1 (*TaIPK1*) reduces phytic acid and improves iron and zinc accumulation in wheat grains (Ibrahim et al. 2022). Pre-harvest sprouting (PHS) refers to the premature germination of grains in the spike before harvesting. In wheat, PHS leads to the deterioration of flour quality due to starch breakdown occurring in the germinated grains. Red-grained wheat varieties are usually more tolerant toward PHS than white-grained wheat varieties (Vetch et al. 2019). The *Tamyb10*, a gene with pleiotropic effects, is also associated with PHS tolerance of grains. The restoration of Tamyb10 using CRISPR/Cas9 is a possible solution to make wheat resistant to pre-harvest sprouting (Zhu et al. 2023). The *Photoperiod-1* (*Ppd-1*) gene in wheat is to regulate flowering time in response to day length or photoperiod and yield in wheat. The spike architecture and grain morphometric traits in wheat are altered through the CRISPR/Cas9 editing of *Ppd-1* gene homoeologs (Errum et al. 2023). *TaDCL4*, *TaDCL5*, and *TaRDR6* are genes found in wheat that are vital for RNA interference (RNAi) processes and gene expression regulation. CRISPR/Cas9-targeted mutagenesis of *TaDCL4*, *TaDCL5*, and *TaRDR6* in common wheat leads to male sterility induction (Zhang et al. 2023). *Triticum aestivum Squamosa Promoter-Binding Protein-Like 13* (*TaSPL13*) is a gene that belongs to the SPL family of transcription factors. Its main function is to regulate flowering time and various developmental processes in wheat (Li et al. 2020). CRISPR/Cas9-induced miRNA156-recognition element mutations in *TaSPL13* lead to improve multiple agronomic traits in wheat (Gupta et al. 2023). The *TaASN2* gene in wheat is related to nitrogen metabolism. *TaASN2* encodes an enzyme called asparagine synthetase, which plays a critical role in the assimilation of nitrogen in the form of asparagine (Raffan et al. 2021). *TaASN2* had been knocked out using CRISPR/Cas9 to reduce asparagine levels in wheat (Raffan et al. 2023). *γ-Gliadin* genes are related to their role in the formation of gluten proteins in wheat. Gluten is composed of glutenins and gliadins and determines the viscoelastic properties of dough and end-use quality in wheat (Saini et al. 2023). The utilization of CRISPR-Cas9 technology to edit the γ-gliadin gene has been shown to enhance end-use quality in wheat (Liu et al. 2023a). The *TaARF15-A1* gene is to act as a negative regulator of senescence. *TaARF15-A1* knockout mutants showed accelerated leaf senescence and grain ripening using CRISPR-Cas9 (Li et al. 2023). The application of CRISPR-based editing on the ω- and γ-gliadin gene clusters results in a reduction of wheat immunoreactivity, while maintaining grain protein quality (Yu et al. 2023).

CRISPR/Cas9 for enhancing biotic stress tolerance of wheat

Biotic stress in plants may be attributed to various living creatures, including fungi, viruses, insects, nematodes, spiders, and weeds (Kumar and Nautiyal, 2022) (Fig. 5). Biotic stress agents deprive the host of nourishment, resulting in diminished plant vigor and, in severe situations, even death of the host. Biotic stress contributes to pre- and postharvest agricultural losses (Shlibak et al. 2021). Only fifty of the almost two hundred diseases and pests that have been identified are regarded as economically significant because they can cause harm to crops and have an effect on the earnings of farmers (Randhawa et al. 2019). Wheat is susceptible to a wide variety of diseases, the most common of which are stripe rust, stem rust, leaf rust, powdery mildew, and head blight (Sabouri et al. 2022). CRISPR/Cas9 technology has recently improved plant traits, including disease resistance (Chen et al. 2019).

The head blight caused by the fungus *Fusarium* is a significant economic factor in wheat, barley, and maize because it reduces crop output and degrades grain quality (Bahadoor et al., 2018). Deoxynivalenol (DON) is a mycotoxin that helps the *Fusarium graminearum* fungus grow in the floral

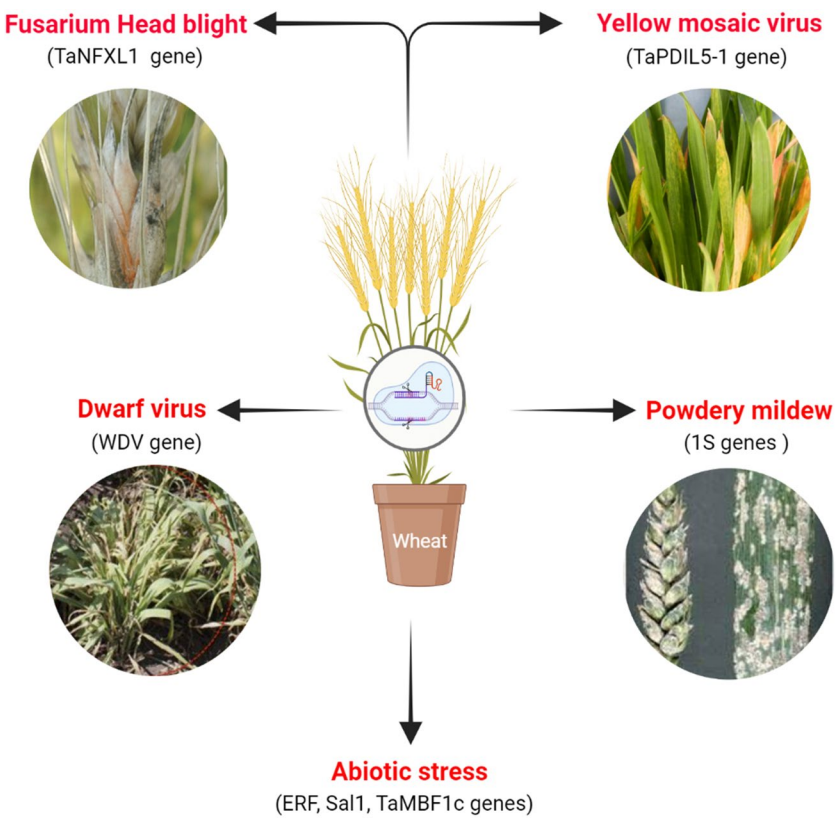

Fig. 5 The application of CRISPR/Cas9 gene editing technology targets specific genes to improve resistance against biotic stressors such as *Fusarium* head blight, wheat yellow mosaic virus, powdery mildew disease, and wheat dwarf virus. Additionally, CRISPR/Cas9 enables the modification of genes related to abiotic stress tolerance, such as salt, drought, and heat

tissues of wheat (Brauer et al. 2020). DON is also a plant toxin that promotes the transmission of pathogens across tissues by causing tissue bleaching, necrosis, and defense-associated cellular responses (Brauer et al. 2020). Some genes, like *TaNFXL1*, were turned on by treating tissues directly with DON (Brauer et al. 2020). According to CRISPR/Cas9-mediated genome editing, targeting the *TaNFXL1* gene may help develop disease resistance (Brauer et al. 2020).

Wheat yellow mosaic virus is a disease-causing agent transmitted via the soil by a fungus-like creature known as *Polymyxa graminis* (Zhang et al. 2021a). Moreover, the illness causes the leaves to become striped with yellow and causes the plant to develop more slowly, leading to a significant yield reduction (Holtz et al. 2017). *TaPDIL5-1* demonstrated minor dose effects on the yellow mosaic virus (Kan et al. 2022). Wheat yellow mosaic virus resistance was introduced into hexaploid wheat using the simultaneous editing of the host factor gene *TaPDIL5-1* homoeoalleles (Kan et al. 2022).

CRISPR/Cas9 was used for mildew resistance locus O (*TaMLO*) knockout and has been shown to confer wheat resistance to powdery mildew disease caused by *Blumeria graminis* (Wang et al. 2014). *Fusarium* head blight, produced by *Fusarium graminearum*, leaf rust, induced by *Puccinia triticina*, and stripe rust, caused by *Puccinia striiformis*, are problematic fungal diseases globally. *Fusarium* head blight may contaminate grain with mycotoxins, reducing food and feed safety (Ghimire et al. 2020). Recent research indicates that pests and illnesses account for 21.5% of wheat yield losses (Savary et al. 2019). Thirty-three genetic factors, known as *S* genes, were identified as negative regulators, suggesting that disease resistance might be increased by downregulating, deleting, or silencing these genes (Taj et al., 2022). Thirty-three genetic factors are possible CRISPR/Cas9 knockdown targets to increase wheat disease resistance (Taj et al. 2022). Wheat dwarf virus is a phloem-limited virus spread by insects and is of significant economic importance (Tholt et al. 2018). Wheat dwarf viruses cause yield reductions in wheat and barley (Nancarrow et al. 2021). The CRISPR/Cas9 method may create effective wheat dwarf virus resistance in monocotyledonous plants (Kis et al. 2019).

The *TaPDIL5-1* encodes protein disulfide isomerase-like 5-1. It belongs to the family of protein disulfide isomerases (PDIs), which are involved in protein folding, assembly, and disulfide bond formation in the endoplasmic reticulum (Kan et al. 2023). CRISPR/Cas9 editing of the TaPDIL5-1 gene confers wheat yellow mosaic virus resistance in wheat (Kan et al. 2022). The *TaCIPK14* gene encodes a protein that belongs to the CBL-interacting protein kinase (CIPK) family. CIPKs are essential components of signal transduction pathways. *TaCIPK14*, as a specific member of this family in wheat, contributes to stress resistance pathogen attacks (Liu et al. 2023b). CRISPR/Cas9-mediated gene knockdown of

TaCIPK14 significantly increased wheat resistance to stripe rust in wheat (He et al. 2023).

CRISPR/Cas9 for enhancing abiotic stress tolerance of wheat

The abiotic stressors include heavy metals, salt, drought, nutritional inadequacy, intense light, pesticide contamination, and severe temperatures (Sharma et al. 2020) (Fig. 5). These stressors impose significant restrictions, which lower agricultural output and threaten food security around the globe (Neupane et al. 2022). Abiotic stressors impair plant photosynthetic efficiency by affecting chlorophyll production, photosystem performance, electron transport, gas exchange, and other factors (Sharma et al. 2020). Cereals, such as wheat, rice, and maize, are among the most widely grown crops because they provide a primary source of calories and protein (Tack et al. 2015).

Ethylene response factors (ERFs) are AP2/ERF superfamily proteins with a DNA-binding domain that contribute to multiple abiotic stress tolerance, such as salt, drought, heat, and cold (Yu et al. 2022). Some ERFs and DREBs function as stress-tolerance repressors that downregulate stress-induced gene transcripts (Yu et al. 2022). Due to the complexity of the wheat genome and the size of the *AP2/ERF* family, *AP2/ERF* members are numerous in wheat and have various activities. It is challenging to swiftly and uniquely identify abiotic stress-related *AP2/ERF* genes (Debbarma et al. 2019). *ERF* genome editing uses CRISPR/Cas9 to improve crop tolerance to multiple abiotic stresses (Debbarma et al. 2019).

The *Sal1* encodes 3′(2′),5′-bisphosphate nucleotidase and inositol polyphosphate 1-phosphatase (Mohr et al. 2022). *Sal1* inhibits drought tolerance, and the *Sal1* mutant allele increases inositol phosphate, ABA, and stress gene expression (Wilson et al. 2009). ABA increases the closing of stomata in plant guard cells to save water and causes alterations in gene expression and adaptive physiological reactions (Ram et al. 2020). *Sal1* gene silencing in wheat enhances drought tolerance (Abdallah et al. 2022). The *TaMBF1c* gene is a member of the multiprotein bridging factor 1 (MBF1) family. This gene family is known for its role in cellular stress responses. In wheat, the *TaMBF1c* gene plays a crucial role in response to abiotic stresses. Studies have found that it is upregulated under conditions such as drought, high salinity, and cold, which are major environmental factors affecting the growth and productivity of wheat (Tian et al. 2022). Overexpression of the *TaMBF1c* gene in wheat enhances its tolerance to these abiotic stresses, promoting better growth and productivity under adverse conditions. Therefore, understanding and manipulating this gene can have significant implications for improving wheat crop performance (Yadav et al. 2022).

We have summarized the recent applications of CRISPR/Cas9-mediated gene editing in wheat in Table 1.

Table 1 Summary of the recent applications of CRISPR/Cas9-based genome editing in wheat.

Cultivar	Target gene(s)	Gene function	Delivery mode	Reference
Applications of CRISPR/Cas9 for enhancing yield quality of wheat				
Bread wheat (*T. aestivum* L.)	*Pinb gene*	Controls grain hardness	*A. tumefaciens*-mediated transformation	Zhang et al. (2021c), Zhang et al. (2018)
Bread wheat (*T. aestivum* L.)	*Wx genes*	Key enzyme in amylase biosynthesis	*A. tumefaciens*-mediated transformation	Zhang et al. (2021c)
Bread wheat (*T. aestivum* L.)	*TaSBEIIa gene*	Determining starch composition	Biolistic transformation	Li et al. (2021b)
Bread wheat (*T. aestivum* L.)	*Gluten* genes	The synthesis of gluten proteins	Biolistic transformation	Jouanin et al. (2020)
Bread wheat (*T. aestivum* L.)	*TaPSY* genes	Carotenoid production	*A. tumefaciens*-mediated transformation	Zhang et al. (2021c)
Bread wheat (*T. aestivum* L.)	*TaGW7* gene	Controls wheat grain width and weight	*A. tumefaciens*-mediated transformation	Wang et al. (2019)
Bread wheat (*T. aestivum* L.)	*TaARE1* gene	Controls nitrogen use efficiency	Protoplast transformation	Zhang et al. (2021b)
Bread wheat (*T. aestivum* L.)	*DUO-B1* gene	Regulates spike inflorescence	*A. tumefaciens*-mediated transformation	Wang et al. (2022)
Bread wheat (*T. aestivum* L.)	*TaIAA21* gene	Controls wheat grain size and weight	*A. tumefaciens*-mediated transformation	Jia et al. (2021)
Bread wheat (*T. aestivum* L.)	*TaQ* genes	Influence spike evolution	*A. tumefaciens*-mediated transformation	Liu et al. (2020)
Bread wheat (*T. aestivum* L.)	*TaASN* genes	Asparagine synthetase	Biolistic transformation	Raffan et al. (2021)

Bread wheat (*T. aestivum* L.)	*IPK1* gene	Phytic acid biosynthesis	Biolistic transformation	Ibrahim et al. (2022)
White wheat	*Tamyb10* gene	Pre-harvest sprouting-resistant	*A. tumefaciens*-mediated transformation	Zhu et al. (2023)
Bread wheat (*T. aestivum* L.)	*Ppd-1* gene	Regulate flowering and yield	*A. tumefaciens*-mediated transformation	Errum et al. (2023)
Bread wheat (*T. aestivum* L.)	*TaDCL* gene	Production of small RNA molecules	*A. tumefaciens*-mediated transformation	Zhang et al. (2023)
Bread wheat (*T. aestivum* L.)	*TaSPL13* gene	Regulate flowering time and various developmental processes	*A. tumefaciens*-mediated transformation	Gupta et al. (2023)
Bread wheat (*T. aestivum* L.)	*TaASN2 gene*	Encodes asparagine synthetase	Biolistic transformation	Raffan et al. (2023)
Bread wheat (*T. aestivum* L.)	*γ-Gliadin* genes	Formation of gluten proteins	*A. tumefaciens*-mediated transformation	Liu et al. (2023a)
Bread wheat (*T. aestivum* L.)	*TaARF15* gene	Negatively regulates senescence	Biolistic transformation	Li et al. (2023)
Bread wheat (*T. aestivum* L.)	*α/β-Gliadin* genes	Synthesis of gliadins	Biolistic transformation	Yu et al. (2023)
Applications of CRISPR/Cas9 for enhancing biotic stress tolerance of wheat				
Bread wheat (*T. aestivum* L.)	*TaNFXL1* gene	Negatively regulates trichothecene-induced defense responses	Biolistic transformation	Brauer et al. (2020)
Bread wheat (*T. aestivum* L.)	*TaPDIL5-1* gene	Encodes protein disulfide isomerase-like 5-1	*A. tumefaciens*-mediated transformation	Kan et al. (2022)
Bread wheat (*T. aestivum* L.)	*TaMLO* genes	Loss of function confers resistance to powdery mildew	Biolistic transformation	Wang et al. (2014)
Bread wheat (*T. aestivum* L.)	*TaALS, TaAC-Case*genes	The absence of the gene provides herbicide tolerance	Biolistic transformation	Zhang et al. (2019)
Bread wheat (*T. aestivum* L.)	*S* genes	Loss of function improving biotic stress resistance	Biolistic transformation	Taj et al. (2022)
Bread wheat (*T. aestivum* L.)	*WDV* genome site*s*	Resistance against wheat dwarf virus	*A. tumefaciens*-mediated transformation	Kis et al. (2019)
Bread wheat (*T. aestivum* L.)	*eIF4E* gene	Loss of function improves wheat yellow mosaic virus	Biolistic transformation	Kan et al. (2023)
Bread wheat (*T. aestivum* L.)	*TaCIPK14* gene	Encodes a protein that belongs to the CBL-interacting protein kinase	*A. tumefaciens*-mediated transformation	He et al. (2023)
Applications of CRISPR/Cas9 for enhancing abiotic stress tolerance of wheat				
Bread wheat (*T. aestivum* L.)	*TaERF3* gene	Increases salt and drought stress tolerance	Biolistic/*A. tumefaciens*-mediated transformation	Debbarma et al. (2019)
Bread wheat (*T. aestivum* L.)	*Sal1* gene	Enhances drought stress tolerance	Biolistic transformation	Abdallah et al. (2022)
Bread wheat (*T. aestivum* L.)	*TaMBF1c* gene	Enhances heat stress tolerance	*A. tumefaciens*-mediated transformation	Tian et al. (2022)

Opportunities and challenges in CRISPR/Cas9 genome editing

The CRISPR/Cas9 system is a genome editing tool, simple and more robust than traditional methods. Its application accelerates crop improvement (Li et al. 2022). Its multiplexed gene editing ability makes it a preferable breeding tool to improve multiple traits at the same time. CRISPR/Cas9-based crop improvement is a potent plant breeding tool that offers significant advantages over classical breeding. It enables crop improvements in less than a year, in contrast to the 6–7 years typically needed using traditional methods (Hussain et al. 2018). CRISPR/Cas9 genome editing

technology offers exciting opportunities in wheat breeding. Its precise DNA modification capabilities can be utilized to enhance disease resistance, increase yield, improve stress tolerance, and enhance wheat quality (Li et al. 2021c).

The CRISPR/Cas9 system, despite its revolutionary potential, exhibits certain limitations. Unintentional editing of genomic regions resembling the target sequence can occur, resulting in off-target mutations and potential adverse effects (Ahmad et al. 2020). Gene-edited crops have encountered resistance due to concerns about off-target editing, despite the occurrence of such off-target mutations being very low (below 1%) (Graham et al. 2020). Off-target editing occurs when the Cas9 endonuclease mistakenly targets identical sites within the genome, leading to unintended mutations. There is a need to focus on enhancing the predictability of off-target modifications and understanding their potentially harmful effects (Schultz-Bergin 2018). Bioinformatic-based tools are employed to detect potential off-target effects of CRISPR/Cas9-based systems by comparing gRNA sequences with reference genomes. Conducting whole-genome sequencing of CRISPR/Cas9-derived crops is essential for identifying any off-target mutations (Ahmad et al. 2021). The residual presence of Cas in a genetically stable line could lead to unintended mutations that may be toxic. A study conducted on *Arabidopsis* observed the persistence of Cas activity in subsequent generations (T3) (Feng et al. 2016). To address persistent Cas activity, plasmid-free integration approaches can be employed for delivering gRNA and Cas, such as viral-based expression systems and delivering preassembled gRNA: Cas complexes to plant tissues (Ali et al. 2020). The CRISPR/Cas9 gene editing system has a notable limitation that requires a thorough understanding of the gene of interest before effective editing can be achieved (Ahmad et al. 2020). This understanding includes knowing the gene's complete sequence and its potential role in controlling the trait of interest. Researchers must be able to identify the specific arrangement of nucleotides in the gene's DNA and comprehend its function in biological processes (Martin et al. 2016). Gene flow concerns emerge as a significant obstacle to the widespread adoption of CRISPR/Cas9-edited crops. The migration of edited gene sequences or the occurrence of off-target mutations from the CRISPR/Cas9-edited species to wild-type species can potentially lead to adverse environmental consequences. However, no such case has been reported yet (Ahmad et al. 2021). Manipulating polyploid species presents an intricate challenge, exemplified by common wheat, which possesses a vast and complex genome with A, B, and D subgenomes. Attempting to create mutations at multiple genomic sites simultaneously requires sophisticated and precise techniques (Li et al. 2021d). The CRISPR/Cas system could edit multiple genes through gRNA cassettes designed using one or many promoters within a single vector system (Hyun 2020). Utilizing CRISPR/Cas9 in wheat improvement has limitations that include challenges with the hexaploid nature, potential off-target effects, difficulty in efficient delivery to wheat cells, varying regulatory hurdles, and public perception of genetically modified crops. Addressing these limitations is crucial to fully harness the potential of CRISPR/Cas9 for enhancing wheat agriculture and ensuring its widespread adoption (Li et al. 2021d).

Future prospects of CRISPR/Cas9 in wheat production

The use of CRISPR/Cas9 in wheat production holds great promise for enhancing crop yield, quality, and resilience to environmental stressors, pests, diseases, and climate change. Potential advancements include increasing the photosynthetic efficiency of wheat and developing nutritionally superior varieties. In the near future, the conversion of wheat into a variety with higher nitrogen use efficiency (NUE), water use efficiency, and increased rates of photosynthesis could be achieved using CRISPR-Cas technology (Ahmad et al. 2021). As the impacts of climate change worsen, genome-edited wheat holds the potential to play a crucial role in ensuring food security by enabling the development of varieties that can thrive under extreme environmental conditions. However, widespread adoption of this technology may face challenges due to public skepticism, regulatory disparities among nations, and technical limitations of the CRISPR/Cas9 system (Ahmad et al. 2020). Researchers need to develop more efficient delivery methods because the transformation efficiency is still low in plant species with complex genomes, such as wheat and other species. CRISPR-Cas-based gene editing also requires tissue culture for plant regeneration from callus, which is time-consuming and laborious. The process may take several months for crops like wheat or cotton, even with well-established protocols. Therefore, tissue culture-free genome-editing systems, such as delivering gRNA via RNA virus-based systems, are needed (Hyun 2020). CRISPR systems can target any form of genetic information (DNA and RNA) and manipulate it in multiple ways, including knockout, knock-in, gene activation, or repression, base editing, and epigenome engineering (Ali et al. 2015). This contributes to targeting important genes in wheat that help withstand environmental conditions and resist pathogenic biological factors, ultimately leading to the improvement of wheat quality and productivity.

Conclusion

CRISPR/Cas9, the latest advancement in genome engineering, has revolutionized crop breeding. It offers a simple, cost-effective, and robust method for genome editing, leading to significant progress in crop improvement. Though the large genome and complex polyploid nature have hindered the development of wheat genetic engineering and breeding in the past, several powerful tools are now available to advance wheat biology. CRISPR/Cas9 has been widely utilized in diverse wheat breeding programs to improve grain yield, grain quality, disease resistance, and resistance against

abiotic stresses, such as drought, salinity, cold, osmotic, and metal toxicity. Addressing the difficulties of genetic modification in wheat requires sustained dedication and collaboration among scientists, breeders, policy-makers, and the public to ensure the future success and sustainability of genetically improved wheat varieties for global food security.

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Data availability No data was used for the research described in the article.

Declarations

Conflict of interest The authors declare no competing interests.

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