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Genetically Modified Wheat

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ARTICLE ID: 045

Introduction

Cereals are a key component of human diets, providing a significant proportion of the protein and calories consumed worldwide. Wheat (*Triticum aestivum* L.) is one of the major staple food crops grown worldwide on more than 17% of the cultivatable land and produced in a wide range of climatic environments and geographic regions. Wheat is vital crop consumed by humans, provide approximately 20% of our energy needs (calories) and 25% of our protein. Because of Green Revolution in 1970s yield gains in large amount due to the introduction of disease resistant semi dwarf high yielding wheat varieties developed by Dr. N.E. Borlaug and colleagues. Since that time, however, global wheat production has stagnated, and current trends show that yields will not be sufficient to meet growing market demands. According to the United Nations' Food and Agriculture Organization (FAO), 756 million tonnes of wheat produced from 220 million ha of arable land in 2016/2017. Despite this, wheat lags behind other major cereals such as maize and rice, both in terms of yield, and the application of genomic tools for its improvement. In order to feed the population of 9 billion people predicted for 2050, wheat yield should grow by over 60% while still maintaining and/or improving its nutritional characteristics.

To achieve this goal without increasing the area of cultivated land, must be concentrated on key traits related to plant productivity and adaptation to environmental challenges. A deficit in this key staple crop could present a serious threat to global food security, so improved molecular-based breeding and genetic engineering techniques are necessary to break through the current yield ceiling. Plant biotechnology has made significant advancements during the past decade, and offers the opportunities to make step-change increases in crop yield by avoiding losses due to disease, pests, and adverse abiotic conditions

such as drought, salinity, heat, etc, in addition to major advances in novel desirable quality characteristics which are unlikely to be achieved by conventional breeding method. Existing modern breeding efforts now need to be complemented with advanced crop functional genomics, which can provide insights into the functioning of wheat genetic determinants. The available tools for wheat genetic modification provide the experimental means to functionally characterize genetic determinants by suppressing or enhancing gene activities. This knowledge can then be used for targeted improvements tailored to the specific needs of the diverse and changing environments in which wheat is grown across the world. This offers the potential to tackle yield gaps wherever they exist, for a variety of causes, enabling this global crop to finally reach its full potential.

Genetically modified wheat- An overview

Genetically modified wheat has been genetically engineered by using biotechnology, so the direct manipulation of its genome is done. Wheat is a natural hybrid derived from breeding of interspecies. It is theorized that wheat's ancestors (*Triticum monococcum*, *Aegilops speltoides*, and *Aegilops tauschii*, all diploid grasses) hybridized naturally over millennia somewhere in West Asia, to create natural polyploid hybrids, the best known of which are common wheat and durum wheat. As of 2020, no GM wheat is grown commercially, although so many field tests have been conducted, with one wheat variety, Bioceres HB4, obtaining regulatory approval from the government.

The first herbicide tolerant wheat produced by genetic engineering was developed by Monsanto, the MON 71800 event, commercially known as Roundup Ready wheat. A gene from common soil bacterium *Agrobacterium tumefaciens* strain CP4 was introduced to wheat to produce a glyphosate tolerant wheat line. The gene codes for the production of a novel form of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) which functions in the shikimate pathway, a biochemical pathway responsible for the synthesis of aromatic amino acids and other aromatic compounds which are vital for growth and survival. Although studies have proven that this glyphosate tolerant wheat is safe and nutritious, just like the other conventional wheat varieties. Monsanto decided not to introduce Roundup Ready wheat to the market.

Salt-tolerant Wheat

CSIRO Plant Industry researchers have already isolated two salt tolerance wheat genes (Nax1

and Nax2), which came from the old wheat relative *Triticum monococcum*. Both genes inhibit sodium, which can be toxic to plants, by limiting its passage from the roots to the shoots. Based on the field trials conducted in Australia in 2009, the lines with the Nax2 gene produced 25% more yield than those without the gene in saline conditions.

Biofortified Wheat

Wheat is also being developed to be safe for people with celiac disease, which is caused by the consumption of gluten that leads to damage to the small intestine resulting in obstructed absorption of nutrients from food, and hence malnutrition. Washington State University (WSU) is currently conducting experiments using genetic techniques to remove the celiac-causing gliadins in the wheat grain with improved baking quality traits. The variety is also expected to contain more lysine, an essential amino acid that is usually scarce in wheat. The composition of grain and forage from glyphosate tolerant wheat MON 71800 is equivalent to that of conventional wheat.

Modifications in Wheat

Genome targeted engineering using nucleases, such as zinc finger nucleases (ZFNs) and TAL effector nucleases (TALENs), were developed in the late 20th century as innovative tools to generate mutations at specific genetic loci. Nuclease-based mutagenesis depends on induced site-specific DNA double-strand breaks (DSBs), which are either repaired by error-prone nonhomologous end joining (NHEJ), or high-fidelity homologous recombination (HR). The former often results in insertions or deletions at the cleavage site leading to loss-of-functional gene knockouts, whereas the latter leads to precise genome modification. In 2012, the eukaryotic genome editing was revolutionized by the introduction of CRISPR/Cas9 (bacterial Clustered Regularly Interspaced Short Palindromic Repeats) technology. This technology confers targeted gene mutagenesis by a Cas9 nuclease that is guided by small RNAs (sgRNAs) to the target gene through base pairing. Tis is in contrast to the DNA-recognition protein domain that must be specifically tailored for each DNA target in the case of ZFNs and TALENs. Because of its universality and operational simplicity compared to ZFNs and TALEN genome editing systems, CRISPR/Cas9 has rapidly superseded these earlier editing systems and been adopted by the majority of the scientific community. In wheat, the principal applicability of CRISPR/Cas9 was demonstrated in protoplasts and suspension cultures, where multiple genes were targeted in the year following the publication of the original

CRISPR/Cas9 principle. Original methods for plant genome editing rely on the delivery of plasmids carrying cassettes for the coexpression of Cas9 and sgRNA, either by Agrobacterium or particle bombardment. For gene editing in wheat, a Cas9 protein containing one or more signals for nuclear localization is expressed from a codon optimized gene under the control of RNA polymerase II promoters such as CaMV35S or ZmUbi, while the sgRNA is usually expressed from a polymerase III promoter (most commonly, rice or wheat U6 and U3 promoters). One of the additional advantages of the CRISPR/Cas system is its potential for multiplexing, i.e., the simultaneous targeting of several genes with a single molecular construct. Multiple sgRNAs can be introduced either as separate transcription units, or in polycistronic form. In bread wheat, editing has been reported in PEG-transfected protoplasts electroporated microspores and cell suspension cultures transformed by *Agrobacterium*. Edited wheat plants have been regenerated from immature embryos, immature embryo-derived callus, or shoot apical meristems transformed via particle bombardment or Agrobacterium. Recently, protocols for DNA-free editing of wheat by delivering in vitro transcripts, or ribonucleoprotein complexes (RNPs) of CRISPR/Cas9 by particle bombardment, have been developed. The authors claim that these methods not only eliminate random integration of the CRISPR/Cas9 coding DNA elements into the targeted genome, but also reduce of-target effects. Thus, these advances allow for the production of completely transgene free mutants in bread wheat with high precision. The main limitation of these transgene-free protocols is the lack of selection in the transformation and regeneration process. Another optimized delivery system has been developed. Here the authors used replicated vectors based on the wheat dwarf virus (Geminiviridae) for cereal genome engineering. It was shown that, due to increased copy number of the system components, virus derived replicons increase gene targeting efficiency greater than 10-fold in wheat callus cells and protoplasts, compared to the no replicating control. The virus-based CRISPR/Cas9 system also promoted multiplexed gene targeted integration in different loci of the polyploidy wheat genome by homologous recombination Since the advent of the principle of RNA guided nuclease genome editing, a number of additional tools for genome modifications and functional genomics studies have been developed. The DNA binding ability of Cas9 and Cas12 has been used to develop tools for various applications, such as transcriptional regulation and fluorescence-based imaging of specific chromosomal loci in plant genomes.

Another nuclease, Cas13, has been applied to degrade mRNAs and combat viral RNA replication. Orthologues of Cas9 found in other bacterial species such as *Neisseria meningitides Staphylococcus aureus* and *Campylobacter jejuni* have different and more complex adjacent motif (PAM) sequences. These PAM sequences function in their native bacterial hosts to direct the CRISPR/Cas9 complex to the target sequence and not to the CRISPR/Cas9 locus. Although the use of these orthologous Cas9 proteins does limit the available target sequences for genome editing, it also reduces of-target edits. Most recently, systems for targeted base editing in wheat have been established by fusing a cytidine deaminase or adenosine deaminase, to the Cas9 nickase for C/G to T/A or A/T to G/C conversion. In these systems, the efficiency of base editing was enhanced by using a Cas9-based nickase instead of an inactive Cas9. As an example, the base editor Cas9-APOBEC3A was used to edit TaMTL (MATRILINEAL) encoding a sperm-specific phospholipase. Loss of function of MTL triggers haploid induction in maize. Ten base-edited wheat mutants with TaMTL knock-out were identified at a frequency of 16.7%, with three being homozygous for all six alleles without InDels. Functional analysis of wheat mutants with TaMTL knock-out is still to be completed. Other nucleases with similar editing functions to that of Cas9 have been identified. Most notably, Cpf1 possesses both DNase and RNase activity and cleaves DNA to generate four to five bp 5-overhangs, potentially enhancing insertion of DNA sequences by homologous recombination. The Cpf1-based editing system has been successfully applied in plants, but not in wheat at this stage. Although many agriculturally important traits of wheat have been targeted by genome editing, some of the main ones include the following: (i) resistance/tolerance to biotic and abiotic stresses, (ii) yield and grain quality, and (iii) male sterility. (i) The first successful experiment using the CRISPR/ Cas9 system in wheat was editing of TaMLO, a powdery mildew-resistance locus. Powdery mildew diseases caused by Blumeria graminis f. sp. tritici result in significant wheat yield losses, and knock-out of the TaMLO leads to disease resistance. The mutation frequency of TaMLO in protoplasts was 28.5%. Further, described editing of the TaMLO-A1 allele by the CRISPR/Cas9 system and simultaneous editing of three homoeoalleles of TaMLO in hexaploid bread wheat using the TALEN nuclease. The mutation frequency of regenerated TaMLO-edited wheat (5.6%) was similar for both editing methods. More recently, used CRISPR/Cas9 technology to generate Taedr1 wheat lines by simultaneous knock-down of the three homologs of wheat TaEDR1, a

negative regulator of powdery mildew resistance. The mutated plants were resistant to powdery mildew and did not show mildew-induced cell death**.** The lipoxygenase genes, TaLpx1 and TaLox2, attracted attention as potential subjects for gene editing in relation to resistance to Fusarium, one of the most devastating fungal diseases in wheat. Lipoxygenases hydrolyze polyunsaturated fatty acids and initiate biosynthesis of oxylipins, playing a role in the activation of jasmonic acid-mediated defence responses in plants. Silencing of the TaLpx-1 gene has resulted in resistance to Fusarium graminearum in wheat. TaLpx1 and TaLox2 genes were edited in protoplasts with a mutation frequency of 9% and 45%, respectively. Wheat plants with mutated TaLOX2 were obtained with a frequency of 9.5%, of which homozygous mutants accounted for 44.7%. The CRISPR/Cas9 system was also used for editing wheat homolog of TaCer9 (ECERIFERUM9) with the goal to improve drought tolerance and water use efficiency .Mutation of the AtCer9 gene in A. thaliana, encoding an E3 ubiquitin ligase, causes elevated amounts of 18-carbon-length cutin monomers and very-long-chain free fatty acids (C24, C26) in cuticular wax, both of which are associated with elevated cuticle membrane thickness and drought tolerance.

Reason- Why GM wheat is not commercialized

Although yet there is no Genetically Modified (GM) wheat in commerce, the debate around GM wheat has never stopped. Various reasons have been given in the literature to explain why GM wheat has not been adopted by farmers. An affordable segregation and traceability system must be established. Also, farmers may be concerned about the long term economic benefits because of the higher price of Genetically Modified seeds and herbicide treatments. Moreover, they would not want to lose their major export markets, especially Japan and the European Union, where the approval process for new GM crops is slow and consumers are strongly opposed to consuming GM foods. Using the United States as an example, the aggregated total wheat demand (domestic demand plus foreign demand) might shift to the left in a segregated marketing channel (which has to be established for non-GM wheat farmers to sell GM free wheat. Consequently, the welfare impact analysis may be flawed if the leftward shift of wheat demand, which might be the reason that GM wheat has not been commercialized, is not taken into account. If a leftward shift of the demand curve could cause aggregate welfare changes for both producers and consumers to be negative, the optimal strategy would certainly not introduce GM wheat. GM wheat have been harder than other

commercialized GM crops by providing a background of the regulatory framework regarding Genetically Modified Organisms (GMOs) and development stages of GM wheat.GM wheat from these trials is not permitted to enter commercial human food or animal feed supplies. CSIRO researchers are developing wheat and wheat products that might improve nutritional properties such as glycaemic index. The Regulator has authorised CSIRO to conduct trials involving feeding foods containing GM wheat to animals and people under some licences.

Conclusions and Prospective Developments

Demand for wheat is projected to rise at a rate of 1.6% annually until 2050 as a result of increased population and prosperity. Consequently, average global wheat yields on a per hectare basis will need to increase to approximately 5 tonnes per ha from the current 3.3 tonnes. Bread wheat has very complicated hexaploid genome and, therefore, further progress in breeding of this crop is dependent upon knowledge of functional genomics. Based on the knowledge of functional genomics, plant biologists can alter the structures and functions of selected key-genes through "genetic manipulation". Genetic transformation is a very powerful tool for generating scientific proof of the roles and functions of key-genes. The authors of this review are not in a position to discuss the applications of GM-wheat in world breeding practice, since this is beyond the scope of this review. However, the term "genetic manipulation" is very broad and includes other molecular approaches that generate products that fall outside the traditional definition of "GM". vRNA interference and CRISPR/Cas9 represent modern and very advanced GM technologies that in a growing number of countries, such as USA and Canada, result in products that attract the same level of regulation as the products of traditional breeding techniques. Such "end-product-based" rather than "process-based" regulation, presents a far more favorable environment for the progression of molecular based breeding technologies, which can and should change the future of wheat breeding across the world. However, all advanced methods will remain simply "laboratory tools" if their application is not connected with wheat breeders currently working by traditional methods. Terefore, we see th chance for real progress and positive future prospects through efective collaborations between plant molecular geneticists and wheat breeders. Te application of novel methods an analysis of genetically manipulated wheat plants for their utility in breeding can be translated through the introgression of genetic traits into conventional wheat breeding programs.