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Wheat genome editing expedited by efficient transformation techniques: Progress and perspectives☆

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ABSTRACT

Genome editing is one of the most promising biotechnologies to improve crop performance. Common wheat is a staple food for mankind. In the past few decades both basic and applied research on common wheat has lagged behind other crop species due to its complex, polyploid genome and difficulties in genetic transformation. Recent breakthroughs in wheat transformation permit a revolution in wheat biotechnology. In this review, we summarize recent progress in wheat genetic transformation and its potential for wheat improvement. We then review recent progress in plant genome editing, which is now readily available in wheat. We also discuss measures to further increase transformation efficiency and potential applications of genome editing in wheat. We propose that, together with a high quality reference genome, the time for efficient genetic engineering and functionality studies in common wheat has arrived.

Contents

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1. Introduction

The challenge of feeding a global population of 9 billion by the middle of this century is enormous [1]. Common wheat as a staple food crop will play a major role in meeting this challenge. Wheat has lagged behind other major cereal crops in development of genetic engineering and biotechnology because of its huge genome, high number of repetitive DNA sequences, hexaploid composition, and low regeneration following genetic transformation [2]. No transgenic wheat has been commercialized and new wheat varieties are mainly developed by conventional breeding techniques that are costly and time-consuming [3]. The key reasons for this is lack of a high quality reference genome sequence and difficulty in transforming wheat.

Plant transformation with exotic genes using vectors like *Agrobacterium* has been the first step in introducing genes of interest to plant cells that must be regenerated into plants that produce normal seeds. Common wheat as a hexaploid plant is one of the most difficult crops to be transformed. Recently, a new technique called PureWheat that significantly improves transformation efficiency was invented by the Japan Tobacco Company [4]. This technique brings the hope of genetically manipulating wheat in a more efficient and diverse manner. It also enables application of new genome editing technologies that are applicable to wheat.

Genome editing as a recently developed technology enables precise manipulation of specific genomic sequences, and will possibly supersede traditional random mutagenesis methods in plant breeding. In general genome editing technologies involve three types of sequence-specific nucleases (SSNs), namely zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeat-associated endonucleases (CRISPR/Cas). Such technologies have versatile functions including targeted gene knock-out and knock-in, gene replacement and activation, and DNA repair [5–8], and will be widely applied in crop breeding. This is likely to be led by the application of CRISPR/Cas9 with the help of plant regeneration-related genes such as *Baby boom* (*Bbm*) and *Wuschel* (*Wus2*) in co-transformation [9].

In this review, we provide a brief summary of current transformation techniques and recent breakthroughs in genetic engineering of wheat. We then review recent progress in plant genome editing and its application in wheat. Finally, we speculate future trends in wheat genetic engineering with the availability of a high quality genome sequence, a significantly improved transformation protocol, and a tool for genome editing in generating elite wheat varieties that will contribute to achievement of world food security.

2. Wheat transformation - possible after much effort

2.1. Transformation of wheat by biolistic particles

The first transgenic wheat plants were obtained by biolistic particle bombardment in 1992 [10]. The *Bar* gene as a selective marker was successfully transferred to wheat by high velocity microprojectile bombardment. This was the beginning of the era of wheat transformation. A number of genes were transformed into wheat using this approach (Table 1), including functional genes such as *TaPIMP1* [20], *Yr10* [22], *TcLr19PR1* [23], *TaNAC2* [26], and *TaCPK* [27]. Genetically enhanced wheat lines that have better resistance to biotic and abiotic stresses are still being tested. However, use of biolistic particles is notorious for its low transformation efficiency.

2.2. Wheat transformation by *Agrobacterium*

Agrobacterium species used for wheat transformation include *A. tumefaciens* and *A. rhizogenes*. They use a transfer DNA (T-DNA) that naturally integrates into plant genomes after infection. *Agrobacterium* mediated transformation has specific advantages compared to biolistic particles, including low copy number integration, a more economic and simpler procedure, and clear integration of sequences without the vector backbone. Although it is widely used by wheat scientists (Table 2), the efficiency of *Agrobacterium* mediated wheat transformation using immature embryos remained extremely low until recently, when the PureWheat technique was developed by the Japan Tobacco Company. This revolutionary technique involved cultivar Fielder as a host with various modifications in transformation protocols. The efficiency of this method has reached as high as 50% [4]. The technique was confirmed in Australia using wheat cultivars Westonia and Gladius [40]. With additional modifications the present authors' laboratory has transformed more than 15 Chinese genotypes, including elite varieties Jimai 22, Shiluan 02-1, Yangmai 16, Jimai 5265, Zhoumai 18, and Lunxuan 987, with high *Agrobacterium* infection efficiency and less genotype dependence (Fig. 1) [39].

From our experience and that of others, a number of factors need to be considered in order to achieve high transformation efficiency. Firstly, the infection efficiency should be high. Wheat genotypes differ in susceptibility to *Agrobacterium* infection. Secondly, the wheat genotype should have high regeneration ability, as exemplified by Bobwhite, Fielder, Kenong 199, and Yangmai 158 [4,26,31,41]. Thirdly, the plants from which immature embryos are collected should be

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