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REVIEW

Current status and trends of wheat genetic transformation studies in China

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Abstract

More than 20 years have passed since the first report on successful genetic transformation of wheat. With the establishment and improvement of transformation platform, great progresses have been made on wheat genetic transformation both on its fundamental and applied studies in China, especially driven by the National Major Project for Transgenic Organism Breeding, China, initiated in 2008. In this review, wheat genetic transformation platform improvement and transgenic research progresses including new techniques applied and functional studies of wheat quality, yield and stress tolerant related genes and biosafety assessment are summarized. The existing problems and the trends in wheat transformation with traditional methods combined with genomic studies and genome editing technology are also discussed.

Keywords: genetic modification, quality, stress tolerance, transformation platform, wheat

1. Introduction

Wheat is counted the third among three big cereal crops in the world, and its yield has increased from 560 million t in 2003 to 713 million t in 2013 (FAO 2013). Since 1991, China has been the top wheat producer in the world, and its wheat production was 121 million t in 2013. Wheat provides staple food for over one-third of the human beings and supplies approximately 20% of the calorie intake (Vasil 2007). It also supplies essential vitamins and minerals. According to the prediction by International Food Policy Research Institute (IFPRI) , the world wheat demand will reach 841 million t in 2020, the requested increase of wheat yield in 2020 is roughly equal to 2013 China's total wheat yield (Rosengrant *et al*. 1995). In view of the current declining arable land, global climate change and sustained growth of the population, wheat production is still facing with great challenges.

Genetic modification (GM) technology has been used to develop many varieties of crops and it is also a powerful tool to study gene function as well. Over the past two decades, GM technology has been successfully used to develop new crop varieties with improved productivity, quality and stress tolerance. The planting area of GM crops has increased from 1.7 million ha in 1996 to 175 million ha in 2013, with an annual growth rate of more than 31.3% (James 2013).

Despite its importance, the progress in wheat genetic transformation still lags behind other major cereal crops such as rice, due to the difficulties associated with gene delivery and recovery of transgenic plants. The first transgenic wheat

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with herbicide resistance was obtained by Vasil *et al*. in 1992 using biolistic approach, and the transformation frequency was only 0.2%. After that, the wheat genetic transformation technology has remarkably improved, and the average of transformation frequency has increased to more than 1%, and even achieved 16.7% (Ye *et al*. 2011). In China, as early as in 1993, transgenic wheat was obtained by laser micro-beam method (Wang *et al*. 1993). Xi *et al*. (2004a) obtained transgenic wheat using the pollen tube pathway method. Since then, reports on GM wheat were gradually increasing in China. In 2008, the Chinese government initiated the National Major Project for Transgenic Organism Breeding, China with the aim to breed new varieties of GM wheat, cotton, maize, rice and soybean etc. with improved agronomic traits. Under the support of this project, the GM wheat research in China has been greatly promoted. In recent years, researchers in China have focused their studies on the establishment of wheat transformation platform with large scale and high efficiency, and the cultivation of GM wheat with improved quality and tolerant to bio- and abio-stresses. This paper reviews the progress of wheat genetic transformation studies in China and highlights research using transgenic approaches to improve the quality traits in wheat. The problems and trends of wheat transgenic transformation research will also be detailed discussed.

2. Platform of wheat genetic transformation

Methods commonly used in wheat genetic transformation include biolistic-, *Agrobacterium*-, PEG-mediated and pollen tube pathway methods. While the biolistic- and *Agrobacterium*-mediated transformation are the two most widely used methods. Comparing to some plant species, only a few kinds of explant tissues of wheat are suitable for plant regeneration from tissue culture. The most commonly used target tissue is the immature embryo, which is amenable to DNA uptake *via* both biolistic- and *Agrobacterium*-mediated methods and immature embryo could easily form embryogenic callus and regenerate into transgenic plantlets. However, there are strict sampling time limit and seasonal restriction on the use of immature embryo (scutellum) as explant. Some laboratories began to use the mature embryos as the explants in wheat genetic transformation. A few other explants such apical point of buds and young spikes were also reported.

2.1. Biolistic-mediated transformation

The principle of biolistic is that high-speed metal particles penetrate across the cell wall, and then the DNA coated on these particles are released and randomly integrated into the plant genome. Biolistic method is less genotype dependent, and is generally more efficient. However, the DNA integration events are more complex and often result in more transgene copies (Sparks and Jones 2009). Even though, biolistic method is still widely used in most of laboratories in China. Significant progress has been made in methodology development of biolistic method in Chinese laboratories. Publications on methodology improvement of wheat biolistic-mediated transformation in China are listed in Table 1.

Efficiency of biolistic-mediated transformation is mainly affected by bombardment parameters (bombardment distance, helium pressure and vacuum conditions, etc.), donor materials (explant type, pre-culture treatment, and genotype), selective agents (type and concentration) and gold particles (size and dosage). The size of particle influences its ability to penetrate the cell wall and bombardment pressure and distance are related to the penetrability of particle. In addition, bombardment distance affects the degree of bombardment spread (Yao *et al*. 2007; Dong *et al*. 2009). Increasing the target distance tends to reduce the

Table 1 Methodology development for biolistic-mediated wheat transformation in China

Wheat genotype	Explant[1)]	DNA	Transformation frequency (%)[2)]	Reference
Zhong-60634	IE, PCIE	pAHC25	0–0.88	Liang *et al.* (1999)
Yangmai 18, 34, 70, Wenmai 6	IE, PCIE	pBluescript SK$^+$	0–1.28	Ren *et al*. (2004)
96G4, 3383, Xu 541, etc.	YS, IE	pAHC25-DREB1A	0–5.36	Zhao L S *et al*. (2006)
Emai 12	IE	Gene cassette	0.2–0.6	Yao *et al*. (2006)
Emai 12	IE	Gene cassette	0.4–1.1	Yao *et al*. (2007)
Emai 12, Bobwhite	IE, ME	pCal-neo	0.3–0.9	Ding *et al*. (2007)
Shi 4185	APB	pABH9, pUbigus	0.6	Dong *et al.* (2009)
Kenong 199	IE	pAHC25	0–2.45	Min *et al*. (2013)
Nongda 408, Baofeng 104, etc.	ME	pAHC25	1.35–1.8	Li *et al*. (2013)
Longchun 23	IE	pUBI	ND	Cai *et al*. (2013)
Xindong 26	IE	pAHC25 gene cassette	0.4	Qin *et al*. (2014)
Kenong 199, Jimai 22	IE	Gene cassette	0.49–1.86	Su *et al*. (2014)

[1)] IE, fresh immature embryos; PCIE, pre-cultured immature embryos; YS, young spikes; APB, apical point of buds; ME, mature embryos.
[2)] ND, not determined.
The same as below.