Biochemical and Biophysical Research Communications xxx (2018) 1–4

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Stable plastid transformation of rice, a monocot cereal crop

Yunpeng Wang, Zhengyi Wei, Shaochen Xing*

Institute of Agricultural Biotechnology, Jilin Academy of Agricultural Sciences, Changchun, 130033, PR China

ARTICLE INFO

Article history:
Received 25 June 2018
Accepted 28 June 2018
Available online xxx

Keywords:
Rice
Plastid transformation
Green fluorescent protein
Homoplastomic selection

ABSTRACT

Rice is one of the most important cereal crops and its biotechnology has been pursued to meet the food demand of ever-growing global population. Rice plastid transformation has been a great challenge to achieve homoplastomic plants due to its low efficiency of regeneration. In this experiment, Japonica rice line 19 was chosen to be the receptor for plastid transformation. A vector harboring *smGFP* gene was constructed and transferred into rice plastid genome by bombardment. The resistant callus was obtained after long-lasting multiple selections and proved to be in homoplastomic status by molecular testing. The plantlet was regenerated from homoplastomic callus and grown to seeding stage. This is the first case so far to achieve the homoplastomic rice and will be helpful to transform plastid genome of monocotyledonous crops with recalcitrant nature.

1. Introduction

Plastid transformation has achieved striking implementation since its first accomplishment in a unicellular green alga, *Chlamydomonas reinhardtii* [1], followed by stable plastid transformation in tobacco, a well-known model flowering plant [2]. Hitherto, a total of 18 species of flowering plants have been reported to obtain the homoplastomic plants via stable plastid transformation, including the recent study addressing the stable plastid transformation in *Scoparia dulcis* L., a valuable medicinal herb of Plantaginaceae [3].

However, no agronomically important monocot cereal crops such as wheat (*Triticum aestivum*) and maize (*Zea mays*) have been reported to obtain the homoplastomic plants via stable plastid transformation. Nonetheless, one study of wheat plastid transformation was retracted in 2012, one year after publication [4], indicating the difficulty of plastid transformation for monocot crops. To date, plastid engineering technology has not been utilized commercially in the field [5].

As one of the critical crops, rice feed comprises 1/3rd of the worldwide population and genetic improvement of rice in yet under investigation with the development of cutting-edge technology. Although rice plastid transformation was reported before [6,7], no homoplasmy was achieved. In the present study, mature seeds of Japonica rice were used as explants to induce proper callus, and the expression vector harboring the *gfp* was transferred into the plastid genome of rice via biolistic bombardment. The homotransplasmic rice plant was firstly obtained and confirmed by molecular investigation. This preliminary result paves the way for plastid genome manipulation of monocotyledonous cereal crops.

2. Materials and methods

2.1. Rice materials

Three Japonica rice lines, 19, 58 and 808, were selected among the twenty lines depending on the frequency of regeneration. The young panicals of rice were served as explants for callus induction on the induction media. The frequency of regeneration is estimated according to the number of regenerated seedlings per callus. The callus with the highest frequency will be used as the explant for the plastid transformation.

2.2. Induction of callus from young panical of selected rice line

Young panicals, approximately 8–15 mm in length of six lines, were surface-sterilized, cut into 5-mm-long pieces, and placed onto the MS medium supplemented with 2.0 mg/l 2.4-D for callus induction at 25 °C with a 16 h light/8 h dark cycle. The medium was replaced every 20 d until the appearance of callus. The frequency of induction was calculated and the rice line with the highest induction frequency was selected as explant supplier for plastid transformation.

* Corresponding author.
E-mail addresses: wangypbio@cjaas.com (Y. Wang), weizy@cjaas.com (Z. Wei), xingsc@cjaas.com (S. Xing).

https://doi.org/10.1016/j.bbrc.2018.06.164
0006-291X/

2.3. Vector construction, plastid transformation, and homoplastomic selection

Vector construction and plastid transformation of rice were performed as described previously in our lab for alfalfa plastid transformation [8]. The *smgfp* and *HPT* genes were inserted into the appropriate sites of the vector. The callus derived from young panical is the practical approach for plastid transformation.

Briefly, the bombarded callus were placed on the selection medium (MS salt, 30 g/l sucrose and 8 g/l agar, pH 5.8) and cultured for 3 days at 25 °C in the dark. The medium was replaced every 20 days to gradually achieve the homoplastomic status. The PCR test was conducted every two months to investigate the status of callus until the completion of obtaining the homoplastomic callus. During this process, the GFP expression in callus was also checked under UV light to monitor the selection. Confocal fluorescence microscopy was used to confirm GFP expression of in leaves of transplastomic rice according to the protocol described by Wei et al. [8].

2.4. Regeneration and molecular testing of transplastomic plants

The homoplastomic calli were placed on the regeneration medium (MS medium supplemented with 30 g/l sucrose, 8 g/l agar, 0.05 mg/l α-naphthalene acetic acid, 0.25 mg/l 6-benzyl-aminopurine, and 500 mg/l spectinomycin) to obtain the plantlets. Then, the plantlets were moved to another medium for rooting. The plants with roots were moved to pots with soil for growing. Total DNA was extracted from young leaves of the transformed plant for PCR and Southern blotting analysis.

3. Results and discussion

3.1. Selection of suitable rice line for chloroplast transformation

Three lines including 19, 58, and 808 were preliminarily selected depending on their ability of regeneration, and line 19 was finally reconfirmed to be the target line due to the optimal frequency of regeneration than the other two lines. Based on the growing situation of the three lines on the medium, line 19 exhibited the better ability of regeneration than lines 808 and 58 after a prolonged subculture.

3.2. Vector construction and functional identification

The vector was constructed for rice plastid transformation according to the homologous recombinant mechanism. The expression cassette harboring the *smGFP* gene and *hpt* selectable gene, was inserted into the site of rice plastid genome flanking with *16S-trn I* and *trn A-23S* sequences (Fig. 1a).

The presence of *smGFP* gene in the vector was confirmed by using *E. coli* in advance before plastid transformation. Fig. 1b displayed the expression of GFP protein in *E. coli* cells transformed with/without expression cassette in the vector.

3.3. Callus from young panical and plastid transformation and selection

The young panicals were sterilized using ethanol and placed on induction medium supplemented with appropriate resistant antibiotics to obtain the callus. The callus was bombarded with expression vector and placed on recovery medium for 3 days in the

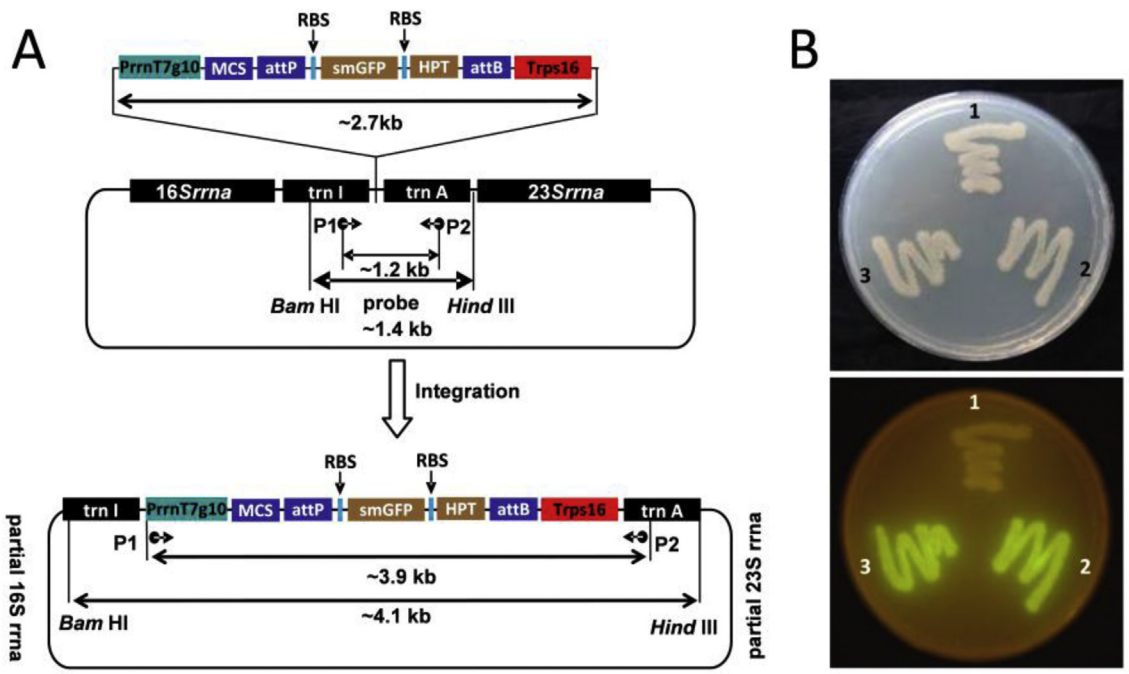

Fig. 1. Construction of expression vector. (**A**) Expression vector for rice plastid transformation was constructed in which the expression cassette containing the multiple cloning site (MCS), *smGFP* gene and *HPT* selectable gene was inserted into the site between *trn* I and *trn* A. These two genes were jointly driven by *Prrn-T7g10* promoter, and the terminator was 3′UTR rps16 (T*rps16*). P1/P2 was designed for PCR analysis and probe was used for Southern blotting. A ~3.9 kb PCR-amplified fragment was expected for the transplastomic plant, however, only a ~1.2 kb fragment was expected for the wild-type plant. Similarly, a ~4.1 kb Southern-hybridized signal was expected for transplastomic plant digested by *Bam* HI and *Hind* III, and only a ~1.4 kb for untransformed plants. (**B**) The photo of upper petri dish was taken under the natural light and lower one was taken under the UV light. No green fluorescence was observed in *E. coli* transformed with vector without the expression cassette (1) while the other two clones 2 and 3 showed clear green fluorescence when transformed with the vector containing the expression cassette. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Please cite this article in press as: Y. Wang, et al., Stable plastid transformation of rice, a monocot cereal crop, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.06.164