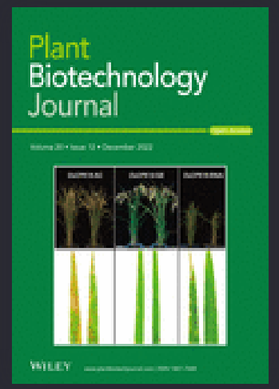
Wiley Online Library

Page 1 / 10

Details



Plant Biotechnology Journal
Volume 20, Issue 12
Dec 2022
Pages 2231-2429

ARTICLE

Multiplexed promoter and gene editing in wheat using a virus-based guide RNA delivery system

View article page

Wei Wang, Zitong Yu, Fei He, Guihua Bai, Harold N. Trick, Alina Akhunova, Eduard Akhunov

CITE

© 2022 The Authors. *Plant Biotechnology Journal* published by Society for Experimental Biology and The Association of Applied Biologists and John Wiley & Sons Ltd.

https://doi.org/10.1111/pbi.13910

ISSN 1467-7644

Plant Biotechnology Journal
Open Access

Plant Biotechnology Journal (2022) **20**, pp. 2332–2341 doi: 10.1111/pbi.13910

Multiplexed promoter and gene editing in wheat using a virus-based guide RNA delivery system

Wei Wang[1,2], Zitong Yu[1,2], Fei He[1,a], Guihua Bai[3], Harold N. Trick[1], Alina Akhunova[1,4] and Eduard Akhunov[1,2,*]

[1]*Department of Plant Pathology, Kansas State University, Manhattan, KS, USA*
[2]*Wheat Genetic Resources Center, Kansas State University, Manhattan, KS, USA*
[3]*Hard Winter Wheat Genetics Research Unit, USDA-ARS, Manhattan, KS, USA*
[4]*Integrated Genomic Facility, Kansas State University, Manhattan, KS, USA*

Received 14 March 2022;
revised 20 July 2022;
accepted 7 August 2022.
**Correspondence* (Tel +1-785-532-1342; fax +1-785-532-5692; email eakhunov@ksu.edu)
[a]Present address: State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China

Keywords: wheat genome editing, barley stripe mosaic virus, promoter editing, multiplex editing.

Summary

The low efficiency of genetic transformation and gene editing across diverse cultivars hinder the broad application of CRISPR technology for crop improvement. The development of virus-based methods of CRISPR-Cas system delivery into the plant cells holds great promise to overcome these limitations. Here, we perform direct inoculation of wheat leaves with the barley stripe mosaic virus (BSMV) transcripts to deliver guide RNAs (sgRNA) into the Cas9-expressing wheat. We demonstrate that wheat inoculation with the pool of BSMV-sgRNAs could be used to generate heritable precise deletions in the promoter region of a transcription factor and to perform multiplexed editing of agronomic genes. We transfer the high-expressing locus of Cas9 into adapted spring and winter cultivars by marker-assisted introgression and use of the BSMV-sgRNAs to edit two agronomic genes. A strategy presented in our study could be applied to any adapted cultivar for creating new *cis*-regulatory diversity or large-scale editing of multiple genes in biological pathways or QTL regions, opening possibilities for the effective engineering of crop genomes, and accelerating gene discovery and trait improvement efforts.

Introduction

Crop improvement using the CRISPR-Cas-based editing relies on understanding the function of genes involved in the regulation of biological processes affecting phenotypic variation. While major advances were made towards linking genes with major agronomic phenotypes in wheat and other crops, and genome sequences facilitated inter-species extrapolation of functional information among related species, the mechanistic basis of most of the traits remains poorly characterized. The advances in gene mapping and large-scale genomic analyses helped to identify a number of quantitative trait loci (QTL) and biological pathways associated with major traits (He *et al.*, 2022). However, the number of candidate causal genes detected in these studies still remained beyond the technical capabilities of existing genomic (Gil-Humanes *et al.*, 2017; Liang *et al.*, 2017; Sánchez-León *et al.*, 2018; Wang *et al.*, 2014; Zhang *et al.*, 2019), they show relatively low editing efficiency and required transformation of a large number of plants or screening of large populations in the next generation of transgenic plants to recover desired mutations (Wang *et al.*, 2018a, 2021). Moreover, the genetic transformation protocols developed for wheat, and for many other crops, are restricted to few varieties showing high regenerative capabilities (Debernardi *et al.*, 2020). This reduces the utility of CRISPR technology for the high-throughput editing of a large number of genes or the direct editing of adapted cultivars for testing the effects of novel CRISPR-induced alleles in diverse genetic backgrounds. The recent discovery of growth regulators *Baby boom*, *Wuschel* (Lowe *et al.*, 2016), and *GRF-GIF* (Debernardi