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Technical Note

Plant-Based Biosensors for Detecting CRISPR-Mediated Genome Engineering

Guoliang Yuan, Md. Mahmudul Hassan, Tao Yao, Haiwei Lu, Michael Melesse Vergara, Jesse L. Labbé, Wellington Muchero, Changtian Pan, Jin-Gui Chen, Gerald A. Tuskan, Yiping Qi, Paul E. Abraham,* and Xiaohan Yang*

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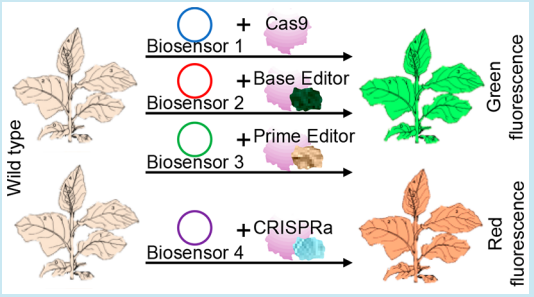
ABSTRACT: CRISPR/Cas has recently emerged as the most reliable system for genome engineering in various species. However, concerns about risks associated with the CRISPR/Cas technology are increasing on potential unintended DNA changes that might accidentally arise from CRISPR gene editing. Developing a system that can detect and report the presence of active CRISPR/Cas tools in biological systems is therefore very necessary. Here, we developed four real-time detection systems that can spontaneously indicate the presence of active CRISPR-Cas tools for genome editing and gene regulation including CRISPR/Cas9 nuclease, base editing, prime editing, and CRISPRa in plants. Using the fluorescence-based molecular biosensors, we demonstrated that the activities of CRISPR/Cas9 nuclease, base editing, prime editing, and CRISPRa can be effectively detected in transient expression via protoplast transformation and leaf infiltration (in *Arabidopsis*, poplar, and tobacco) and stable transformation in *Arabidopsis*.



KEYWORDS: *CRISPR, genome editing, biosensor, detection, transient gene expression*

Different CRISPR/Cas-based genome engineering tools, such as base editors (BEs), prime editors (PEs), CRISPR activation (CRISPRa), and interference (CRISPRi), have been developed recently.[1,2] However, the CRISPR/Cas technology poses potential biosecurity risks, increasing ethical and safety concerns.[3,4] One major concern is that unwanted DNA changes or "off-target" events might accidentally arise from the continuous expression of active CRISPR systems.[5] The unwanted CRISPR/Cas activities can cause an unpredictable phenotypic change in plants, which can negatively affect long-term environmental sustainability and food and animal feed safety, especially in perennial plants with long life-cycle and/or vegetative reproduction. Therefore, it is necessary to develop a system that can detect the presence of active CRISPR/Cas tools in biological systems. Different CRISPR/Cas technologies have been tested in various plant species.[6] However, methods for real-time detection of CRISPR systems have not yet been reported in plants. Given that *Streptococcus pyogenes* Cas9 (SpCas9) is currently the most widely used genome editor in plants, we developed several fluorescence-based biosensors to detect *in planta* activities of SpCas9-based CRISPR/Cas9, base editors, prime editors, and CRISPRa. One advantage of our function-based biosensors is that they do not require any prior knowledge of the DNA sequence for the CRISPR systems, which is a prerequisite for many DNA based detection methods.

RESULTS AND DISCUSSION

To detect active Cas9 nucleases, we generated two variants of biosensor 1 (BS1), BS1-1 and BS1-2, which contain a mutated *GFP* gene (*gfp1a* or *gfp1b*) harboring different frameshift mutations, and single guide RNAs (sgRNAs) targeting *gfp1a* and *gfp1b*, respectively (Figure 1a). When a Cas9 nuclease is introduced and associates with BS1 encoded sgRNA, functional GFP can be generated through CRISPR/Cas9-mediated insertion/deletions in *gfp1a* or *gfp1b* (Supplementary video 1). We coexpressed a T-DNA vector p201N-Cas9[7] with BS1-1/BS1-2 to detect CRISPR/Cas9-mediated gene editing in *Arabidopsis thaliana* protoplasts and *Nicotiana benthamiana* (tobacco) leaves. We observed bright green fluorescence in both the protoplasts transformed with 35Sp:GFP (positive control) and those cotransfected with p201N-Cas9 and BS1-1/BS1-2, but not in the protoplasts transformed with BS1-1/BS1-2 only (negative control) (Figure 1b). Furthermore, ∼34% and ∼15% of the cells exhibited GFP signals in the positive control and the samples with Cas9-BS1 cotransformation, respectively

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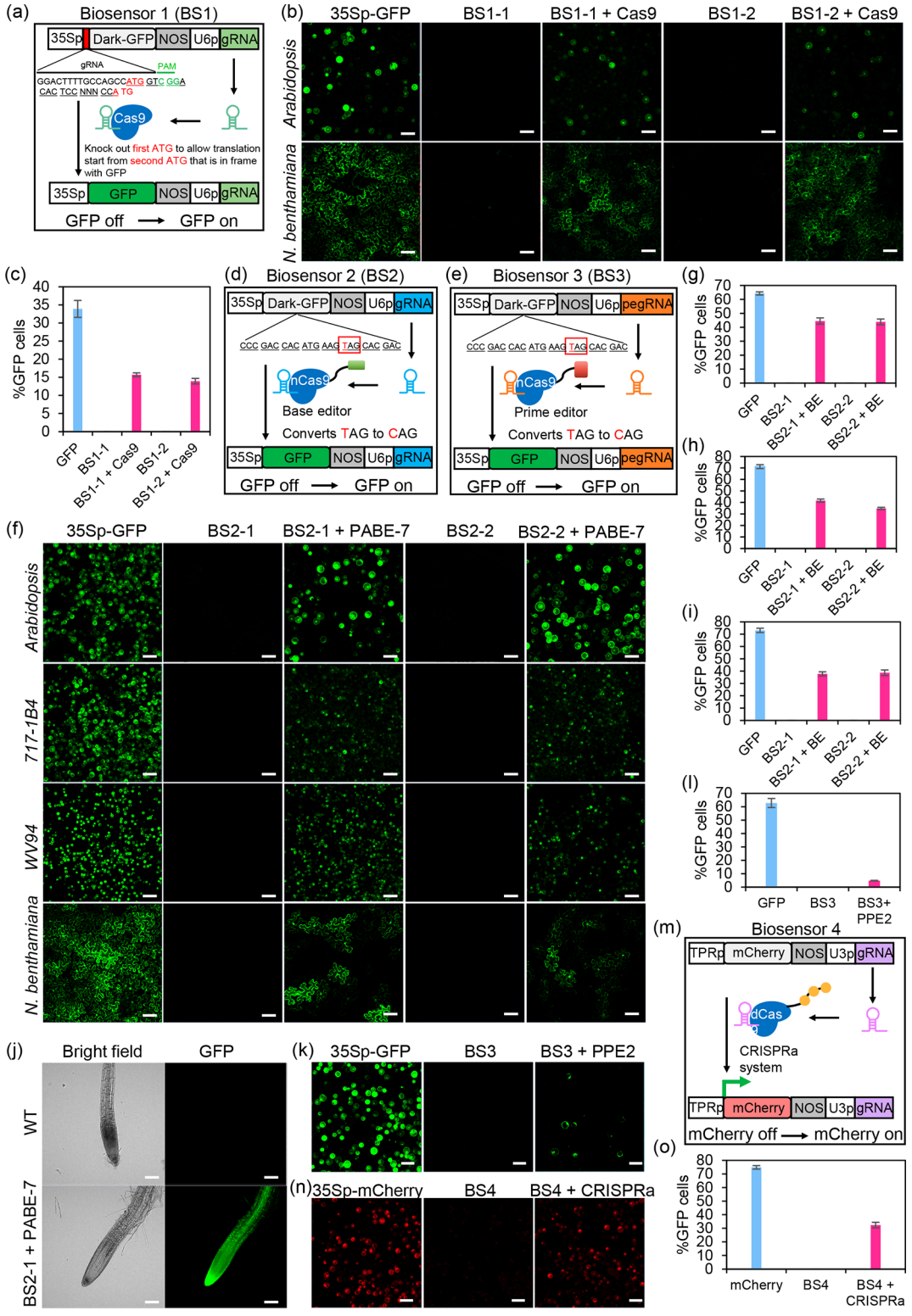

Figure 1. Detection of CRISPR tools for genome editing and gene regulation in plants. (a) BS1 for detection of the CRISPR/Cas9 nuclease. (b) Detection of CRISPR/Cas9 by BS1 through *Arabidopsis* protoplast transformation and tobacco leaf infiltration. (c) Statistical analysis of GFP-positive cells with and without the CRISPR/Cas9 in *Arabidopsis*. (d) BS2 for detection of the adenine base editor (ABE). (e) BS3 for detection of the prime editor. (f) Detection of an ABE by BS2 through protoplast transformation in *Arabidopsis* and poplar, and tobacco leaf infiltration. (g–i) Statistical analysis of GFP-positive cells with and without ABE in *Arabidopsis*, poplar 717-1B4, and poplar WV94. (j) Detection of an ABE by BS2 through stable transformation in *Arabidopsis*. (k) Detection of prime editor PPE2 by BS3 through *Arabidopsis* protoplast transformation. (l) Statistical analysis of GFP-positive cells with and without PPE2. (m) BS4 for detection of CRISPRa. (n) Detection of CRISPRa by BS4 through *Arabidopsis* protoplast transformation. (o) Statistical analysis of mCherry-positive cells with and without the CRISPR–Act3.0 activation system. Scale bar, 100 μm. All data are presented as the mean ± SE (n = 5 independent scopes).

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(Figure 1c). Also, we obtained similar results through *Agrobacterium*-mediated leaf infiltration in tobacco (Figure 1b). These results indicate that BS1 can be used as an efficient biosensor for detecting active Cas9 in plant systems.

To detect active adenine base editors (ABEs) and prime editors, we generated BS2 and BS3 by integrating a dark-GFP mutant (*gfp2*) harboring a premature termination codon (PTC) with corresponding sgRNA and prime editing guide RNA (pegRNA) (Figure 1d,e). As such, an active adenine base editor or prime editor will associate with BS2 or BS3, respectively, to generate a functional GFP protein. We tested two BS2 variants, BS2-1 (*gfp2a*, Q69 > TAG) and BS2-2 (*gfp2b*, Q80 > TAG), by coexpressing with a plant adenine base editor PABE-7[8] using protoplast transformation in *Arabidopsis*, poplar 717-1B4 (*Populus tremula x alba* INRA 717-1B4), poplar WV94 (*Populus deltoides* WV94), and tobacco leaf infiltration. In *Arabidopsis*, poplar 717-1B4 and WV94, we observed strong GFP fluorescence in both the positive control (35Sp:GFP) and the protoplasts cotransfected with PABE-7 and BS2-1/BS2-2 but not those transformed with BS2-1/BS2-2 alone (Figure 1f), indicating that base editing mediated by PABE-7 successfully rescued the *gfp2* mutants. In *Arabidopsis*, ∼64% and ∼45% of the cells exhibited GFP fluorescence in the positive control and the samples with BS2-PABE-7 cotransformation, respectively (Figure 1g). We detected GFP fluorescence in ∼71%, ∼45%, and ∼35% of the poplar 717-1B4 cells in the positive control, the samples cotransfected with PABE-7 and BS2-1, and the samples cotransfected with PABE-7 and BS2-2, respectively (Figure 1h). Also, we detected GFP fluorescence in ∼73% and ∼38% of the poplar WV94 cells in the positive control and the protoplasts cotransformed with PABE-7 and BS2-1/BS2-2, respectively (Figure 1i). These results indicate that both BS2-1 and BS2-2 are relatively efficient for detecting base editing in protoplasts derived from herbaceous and woody plants. Also, we obtained similar results in the tobacco leaf (Figure 1f). Furthermore, after cotransformation of the BS2-1 and PABE-7 using floral dip, we observed GFP signals in the primary root of *Arabidopsis* transgenic lines (Figure 1j). Taken together, results from both transient gene expression and stable transformation demonstrate that biosensor 2 is a reliable and robust system for the detection of ABE-mediated gene editing in plants.

We tested BS3 containing the *gfp2b* mutant and a pegRNA by coexpressing it with a plant prime editor PPE2[9] in *Arabidopsis* protoplasts. We detected GFP signals in the protoplasts with the positive control (35Sp:GFP) and those cotransformed with BS3 and PPE2, but not in the protoplasts transformed with BS3 alone (Figure 1k). This result indicates that the prime editing successfully rescued the *gfp2b* mutant, producing functional GFP proteins. Notably, a relatively low percentage (∼5%) of protoplasts displayed GFP signals in the cotransformation of BS3 and PPE2 in comparison with that (∼63%) in the positive control (Figure 1l). This could be explained by the lower efficiencies of the current generation of prime editors in plants.[2] Therefore, while biosensor 3 can be used to detect active prime editing through protoplast transient expression, the efficiency needs to be improved in the future.

To detect active CRISPRa, we generated BS4 by incorporating the Pro*OsTPR-like*:mCherry and a sgRNA targeting Pro*OsTPR-like*[10] (Figure 1m). As such, we expected that mCherry signals would be much stronger in the presence of a CRISPRa system. We evaluated efficacy of BS4 by coexpressing BS4 and a plant CRISPRa system, CRISPR-Act3.0[10] in *Arabidopsis* protoplasts. We observed strong red fluorescence in ∼75% of protoplasts in the positive control (35Sp:mCherry) and ∼32% of those in the samples cotransformed with BS4 and CRISPR-Act3.0, with no red fluorescence signal detected in the protoplasts transformed with BS4 alone (Figure 1n,o). This result indicates that BS4 is a highly efficient biosensor for detecting CRISPRa activity in plants.

In summary, we developed efficient biosensors for detecting four different CRISPR/Cas tools, providing new opportunities to monitor and evaluate the efficacy of different CRISPR/Cas-based genome engineering tools in real-time. The activities of different CRISPR tools can be detected in the target plants through protoplast transformation, leaf infiltration, or stable transformation. The presence of CRISPR/Cas raises concerns about biosecurity. One of the major challenges in applying CRISPR gene editing technology to agricultural applications is that the gene-edited crop plants need to be transgene-free in order to maintain trait stability and obtain regulatory approval for commercial production.[11] These biosensors may serve as an early detection system for the identification of CRISPR/Cas-free lines in gene-edited crop plants. In addition, these biosensors will aid rapid assessment of these genome engineering systems in various plant species and cultivars. Although these biosensors were designed for detecting SpCas9-based CRISPR tools, they could also be applicable to other Cas proteins with some modifications.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssynbio.1c00455.

Animation depicting how the biosensor detects the CRISPR-Cas9 mediated genome editing in living cells (MP4)

■ AUTHOR INFORMATION

Corresponding Authors

Paul E. Abraham − *Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, United States*; Email: abrahampe@ornl.gov

Xiaohan Yang − *Biosciences Division and The Center for Bioenergy Innovation, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, United States*; orcid.org/0000-0001-5207-4210; Email: yangx@ornl.gov

Authors

Guoliang Yuan − *Biosciences Division and The Center for Bioenergy Innovation, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, United States*; orcid.org/0000-0002-6562-8769

Md. Mahmudul Hassan − *Biosciences Division and The Center for Bioenergy Innovation, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, United States; Department of Genetics and Plant Breeding, Patuakhali Science and Technology University, Dumki, Patuakhali 8602, Bangladesh*

Tao Yao − *Biosciences Division and The Center for Bioenergy Innovation, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, United States*; orcid.org/0000-0002-9382-0731

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Haiwei Lu − *Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, United States;* Present Address: Department of Academic Education, Central Community College—Hastings, Hastings, NE 68902, USA

Michael Melesse Vergara − *Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, United States*

Jesse L. Labbé − *Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, United States;* Present Address: Invaio Sciences, Cambridge, MA, United States.

Wellington Muchero − *Biosciences Division and The Center for Bioenergy Innovation, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, United States*

Changtian Pan − *Department of Plant Science and Landscape Architecture, University of Maryland, College Park, Maryland 20742, United States*

Jin-Gui Chen − *Biosciences Division and The Center for Bioenergy Innovation, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, United States*

Gerald A. Tuskan − *Biosciences Division and The Center for Bioenergy Innovation, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, United States*

Yiping Qi − *Department of Plant Science and Landscape Architecture, University of Maryland, College Park, Maryland 20742, United States; Institute for Bioscience and Biotechnology Research, University of Maryland, Rockville, Maryland 20850, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acssynbio.1c00455

Author Contributions

G.Y., P.A., and X.Y conceived the research. G.Y., T.Y., H.L., and M.H. conducted the experiments. G.Y. wrote the paper. M.H., H.L., T.Y., M.V., J. L., W.M., J.-G.C., G.T., P.A., and X.Y. revised the manuscript. C.P. and Y.Q. provided CRISPRa reagents and revised the manuscript.

Notes

The authors declare no competing financial interest.

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