(SD2022-045) RUBY Plasmids: A reporter for noninvasively monitoring gene expression and plant transformation

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ABSTRACT

Researchers at UC San Diego in collaboration with others have constructed a new reporter RUBY that converts tyrosine to vividly red betalain, which is clearly visible to naked eyes without the need of using special equipment or chemical treatments. They demonstrated that RUBY can be used to noninvasively monitor gene expression in plants. Furthermore, they show that RUBY is an effective selection marker for transformation events.

Reporters have been widely used to visualize gene expression, protein localization, and other cellular activities, but the commonly used reporters require special equipment, expensive chemicals, or invasive treatments.

TECHNOLOGY DESCRIPTION

Researchers at UC San Diego in collaboration with others have constructed a new reporter RUBY that converts tyrosine to vividly red betalain, which is clearly visible to naked eyes without the need of using special equipment or chemical treatments. They demonstrated that RUBY can be used to noninvasively monitor gene expression in plants. Furthermore, they show that RUBY is an effective selection marker for transformation events.

The new reporter will be especially useful for monitoring cellular activities in large crop plants such as a fruit tree under field conditions and for observing transformation and gene expression in tissue culture under sterile conditions.

Subsequently a group researchers in China have developed dual-visible reporter assays to determine the DNA-protein interaction. (Sun H, Wang S, Yang K, Zhu C, Liu Y, Gao Z. Development of dual-visible reporter assays to determine the DNA-protein interaction. Plant J. 2023 Mar;113(5):1095-1101. doi: 10.1111/tpj.16094. Epub 2023 Jan 12.)
https://onlinelibrary.wiley.com/doi/full/10.1111/tpj.16094

And recently in Australia, a team of researchers have developed a split GAL4 RUBY assay

OTHER INFORMATION

KEYWORDS

Plant biotechnology, Genetics, plasmid, gene expression, plant transformation, protein-protein interaction, Ruby, DNA-protein interaction

CATEGORIZED AS

- **Agriculture & Animal Science**
  - Other
  - Transgenics
- **Biotechnology**
  - Food
- **Research Tools**
  - Expression System

RELATED CASES

2021-Z08-1

that enables macroscopically visual PPI detection in plant leaves in real time. Chen, J., Luo, M., Hands, P., Rolland, V., Zhang, J., Li, Z., Outram, M., Dodds, P. and Ayliffe, M. (2023), A split GAL4 RUBY assay for visual in planta detection of protein-protein interactions. Plant J. Accepted Author Manuscript. https://doi.org/10.1111/tpj.16234)

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APPLICATIONS

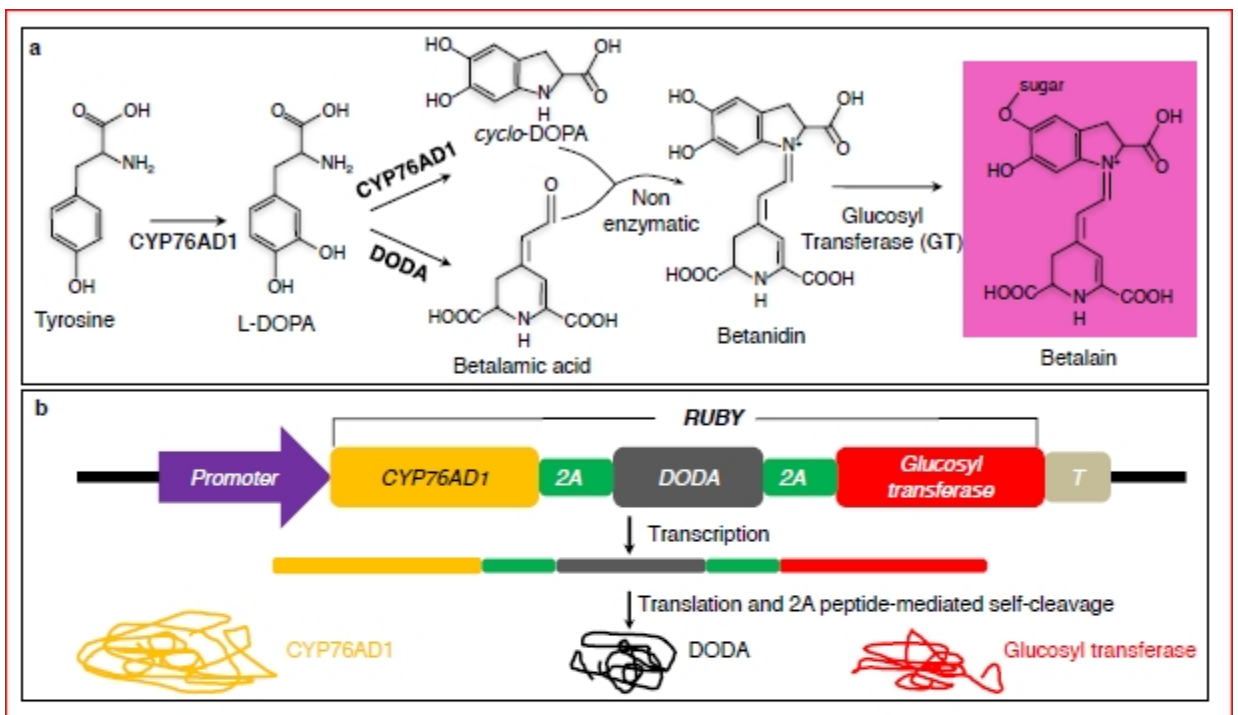


Figure 1. Components required for betalain biosynthesis. a) Chemical reactions for converting tyrosine into betalain, which has a red color. Tyrosine is first oxidized by the P450 CYP76AD1 into L-3,4-dihydroxyphenylalanine (L-DOPA), which can be further converted into *cyclo*-DOPA by P450 CYP76AD1. In the presence of L-DOPA 4,5-dioxygenase (DODA), DOPA is oxidized and circularized into betalamic acid. *Cyclo*-DOPA condenses with betalamic acid, an non-enzymatic reaction, to produce betanidin. Glucoyslastion of betanidin generates the red color betalain. b) A strategy for expressing the whole betalain biosynthetic pathway in a single cassette. The three betalain biosynthetic genes were fused into a single open reading frame, which can be expressed using a single promoter and terminator. Between the genes, sequences that encode 2A peptides were inserted. The 2A peptides undergo self-cleavage, thus releasing the individual enzymes for betalain biosynthesis. The betalain synthesis unit can be placed under the control of a promoter of interest. The terminator used here was the Arabidopsis *HSP18.2* terminator. The open reading frame of 2A linked betalain biosynthesis genes is named *RUBY*.

ADVANTAGES

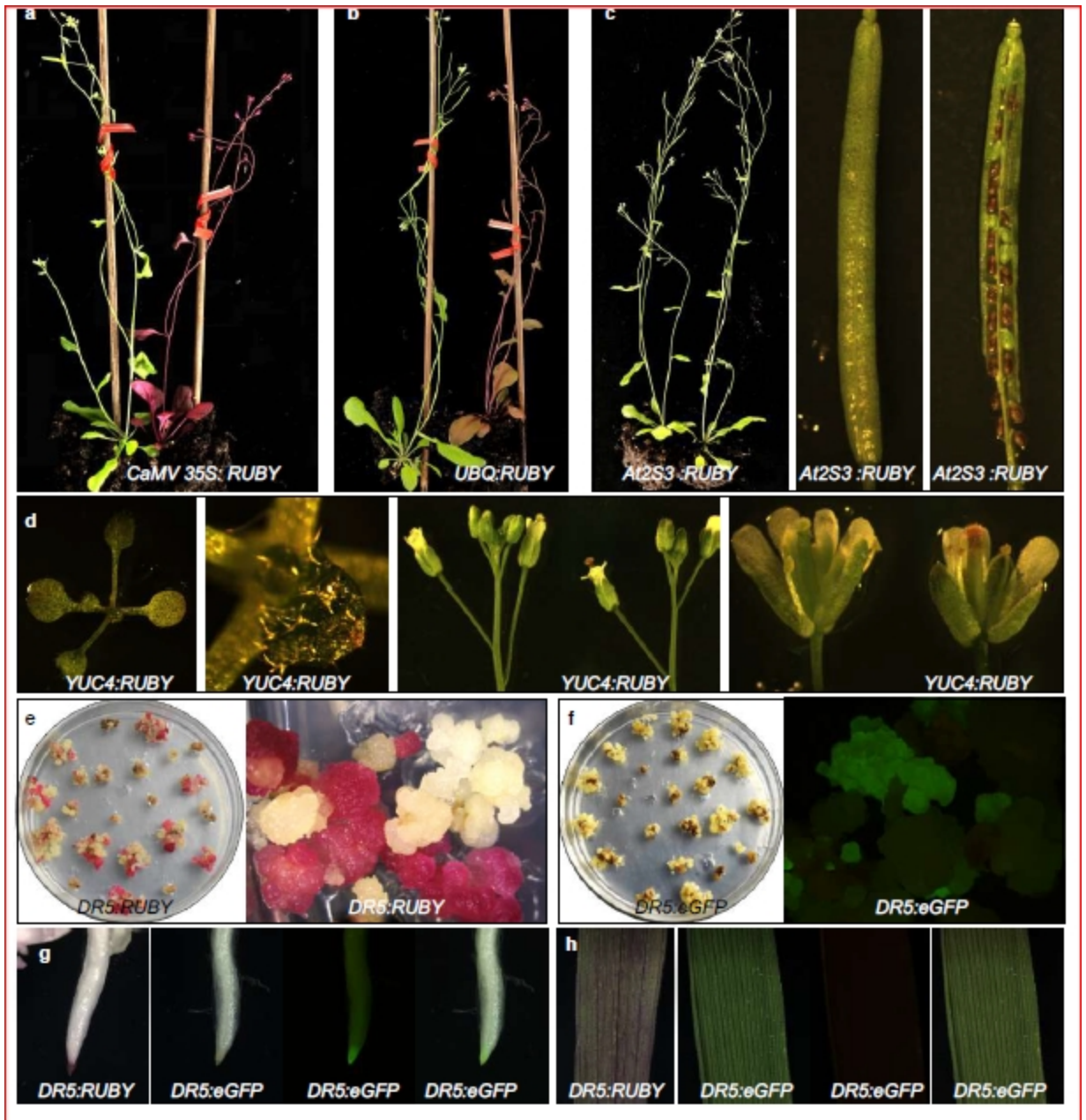


Figure 2. RUBY serves as an effective reporter for gene expression and plant transformation. a) *RUBY* expression driven by the *CaMV 35S* promoter led to a red Arabidopsis plant (right) compared to the WT plant (left). b) *UBIQUITIN* promoter was effective in driving *RUBY* expression throughout the plant (right). Non-transgenic WT was shown left. c) The seed specific promoter *At2S3* did not lead to *RUBY* expression in leaves and stems. The transgenic plant (right) and non-transgenic plant (left) were indistinguishable. The siliques of *At2S3:RUBY* were similar to those of WT, however, when the silique was opened, the seeds of *At2S3:RUBY* were clearly red. Moreover it was obvious that transgenic and no-transgenic seeds were segregating in a silique from a T1 *At2S3:RUBY* plant. d) *YUC4:RUBY* plants displayed patches of red at the tip of leaves and apical region of a gyneocium. e) *DR5:RUBY* was expressed in rice calli. The red color can be used to distinguish transgenic (red) and non-transgenic calli (white). *DR5:eGFP* has been used in rice calli (f), but it was much more difficult to distinguish transgenic from non-transgenic using *DR5:eGFP* compared to *RUBY*. g) Roots of *DR5:RUBY and DR5eGFP* rice plants, which had similar patterns. The three *DR5:eGFP* pictures were generated with the same root: bright field (left), 488 nm fluorescence field (middle), and the merged (right). h) Activation of DR5 promoter in *DR5:RUBY* leaves was easy to observe whereas *DR5:GFP* was much more difficult to detect: bright field (left), 488 nm fluorescence field (middle), and the merged (right).

STATE OF DEVELOPMENT

RUBY plasmid is available for licensing under property rights.

INTELLECTUAL PROPERTY INFO

UC San Diego welcomes requests to provide tangible research materials to interested parties.

RELATED MATERIALS

- He Y, Zhang T, Sun H, Zhan H, Zhao Y. A reporter for noninvasively monitoring gene expression and plant transformation. Hortic Res. 2020 Sep 19;7:152. - 09/19/2020

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