SELECTED TALKS

Problems in detecting activity of fluorescent reporter genes – case of DsRED and GFP

OP1-1

Milica Bogdanović[1], Slađana Todorović[1], Angelina Subotić[1], Milan Dragićević[1], Katarina Cankar[2, 3], Jules Beekwilder[2, 3], Ana Simonović[1]
(milica.bogdanovic@ibiss.bg.ac.rs)

[1] Institute for Biological Research „Siniša Stanković", University of Belgrade, Bul. despota Stefana 142, 11000 Belgrade, Serbia
[2] Laboratory of Plant Physiology, Wageningen University and Research Centre, 6708PB Wageningen, The Netherlands
[3] Plant Research International, Wageningen University and Research Centre, 6708PB Wageningen, The Netherlands

Reporter genes are indispensible tools in plant biotechnology, used to assess transformation efficiency, select transgenic tissues, study promoters, etc. Advantages of fluorescent markers include non-destructive visualization, simplicity of use, non-toxicity, and stability in most cellular contexts. GFP and DsRED are widely used fluorescent reporters and are considered compatible. Unlike animal systems, plant tissues are notoriously known for autofluorescence, spanning most of the visible spectrum. This limits the application of fluorescent proteins in plants and requires special equipment and a set of control samples, to discern autofluorescence from transgene expression. In the course of study of guaianolide biosynthesis in chicory, several vectors were constructed containing *DsRED* as co-transformation reporter and *GFP* as a reporter fused to promoters of the studied genes. To observe DsRED and GFP fluorescence in transgenic chicory tissues, we have used: a system for macro visualization consisting of LED light sources and emission filters, fluorescent stereobinocular, fluorescent microscope and confocal microscope. These methods revealed strong green autofluorescence in newly formed roots, calli, lignified cell walls and parenchyma cells. The intensity of autofluorescence was especially high at high magnification and could obstruct GFP visualization. DsRED fluorescence, on the other hand, was easily discerned from any kind of plant autofluorescence. Another problem was signal crossover from DsRED into GFP channel. DsRED has a small emission peak in the green part of the spectrum, originating from its chromophore maturation. Thus it was impossible to separate fluorescent emission of these two markers present in the same tissue without using spectral deconvolution techniques.

Keywords: DsRED, GFP, LED, fluorescent microscopy, confocal microscopy

This work was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (OI173024) and EU FP7 Project acronym: TERPMED, grant agreement no. 227448.