Characterisation of a *PR-1*::*Luciferase* Transgenic Line Deployed to Uncover Novel Defence-Related *Arabidopsis* Mutants by Luciferase Imaging

A. CHINI, S. MURRAY, J. GRANT, C. THOMSON and G. LOAKE[1*]

Institute of Cell & Molecular Biology, University of Edinburgh, Scotland, United Kingdom; Daniel Rutherford Building, King's Buildings', Edinburgh EH9 3JH, Scotland, United Kingdom

[*]*E-mail: gloake@srv0.bio.ed.ac.uk*

Abstract

In order to identify components of the defence signalling network that may contribute to the establishment of disease resistance, we generated a novel *PR-1*::*Luciferase* transgenic line which was deployed in an imaging based screen to uncover novel defence-related mutants. Approximately, 5 000 ethylmethane sulfonate (EMS) lines and 30 000 activation tagged lines were generated and screened for enhanced LUC activity via ultra low light imaging.

Keywords: signal transduction; luciferase imaging; systemic acquired resistance; *Arabidopsis* mutants

INTRODUCTION

Plants have evolved an extensive array of sophisticated defence mechanisms which are employed to resist the establishment of disease (GLAZEBROOK *et al*. 1997). Pre-formed physical and chemical barriers constitute the first line of defence (OSBOURN 1996), upon which are superimposed a battery of inducible defence responses, including a rapidly activated oxidative burst (GRANT & LOAKE 2000), the cross-linking of cell wall structural components (WALLACE & FRY 1999) and the accumulation of a variety of anti-microbial proteins (BOL *et al*. 1990). The expeditious engagement of these responses is dependent upon successful pathogen recognition, which is thought to occur following the interaction of a specific pathogen avirulence (*avr*) gene product with a corresponding plant resistance (*R*) gene product (DANGL 1995). A near ubiquitous feature of successful pathogen recognition is the visible development of hypersensitive cell death (HR) surrounding the area of attempted pathogen ingress (GREENBERG 1997). Following HR development, is the establishment of immunity throughout the plant to a broad spectrum of ordinarily virulent pathogens. This phenomenon has been termed systemic acquired resistance (SAR) (RYALS *et al*. 1996). The establishment of SAR correlates with a rise in peroxidase activity, increased lignin deposition and the expression of so-called SAR genes, which encode pathogenesis-related (PR) proteins.

MATERIAL AND METHODS

The *PR-1::LUC::OCS* chimeric gene was constructed as described in MURRAY *et al*. (2002). The generated pART27 binary vector was then transformed into *Agrobacterium tumefaciens* strain GV3101. *Arabidopsis* ecotype Col-0 was then transformed by vacuum infiltration and transgenic plants were identified following growth in MS medium containing kanamycin. *Pseudomonas syringae* pv *tomato* DC3000 (*Pst*DC3000) was grown as described in MURRAY *et al*. (2002). Five week old soil-grown plants were infected with a *Pst* DC3000 suspension (OD_{600} = 0.002) in 10 mM $MgCl_2$ by completely infiltrating the abaxial side of the leaf with a 1 ml syringe.

RESULT

In order to accurately determine in real time, in living plant tissue, the temporal and spatial expression programme established by the *PR-1* gene during the

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establishment of *R* gene-mediated resistance and SAR, we generated a transgenic *Arabidopsis* line containing a chimeric *PR-1*::*LUC* gene fusion. The *PR-1* promoter was transcriptionally fused to the firefly luciferase (*LUC*) reporter gene (Figure 1A), and the resulting construct was transformed into *Arabidopsis* accession Col-0. To confirm the utility of a typical transgenic line, the profile of *PR-1*::*LUC* gene expression was compared to that of the endogenous *PR-1* gene following inoculation of *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 expressing the *avrB* gene (Figure 1B). From 12 hours post inoculation, expression of the *PR-1*::*LUC* gene steadily increased, reaching a maximum value between 36 and 48 hours post inoculation, after which time *PR-1*::*LUC* expression slowly decreased. The expression of the endogenous *PR-1* gene was found to be congruent with expression of the *PR1*::*LUC* transgene. We also determined the temporal profile of LUC activity following inoculation with *Pst* DC3000 (*avrB*) (Figure 1C). This also exhibited similar kinetics to that of the endogenous *PR-1* gene, with maximum LUC activity recorded at 36 h post inoculation. These experiments therefore confirmed that the *PR-1*::*LUC* transgene functioned as a robust reporter for the engagement of endogenous *PR-1* gene expression.

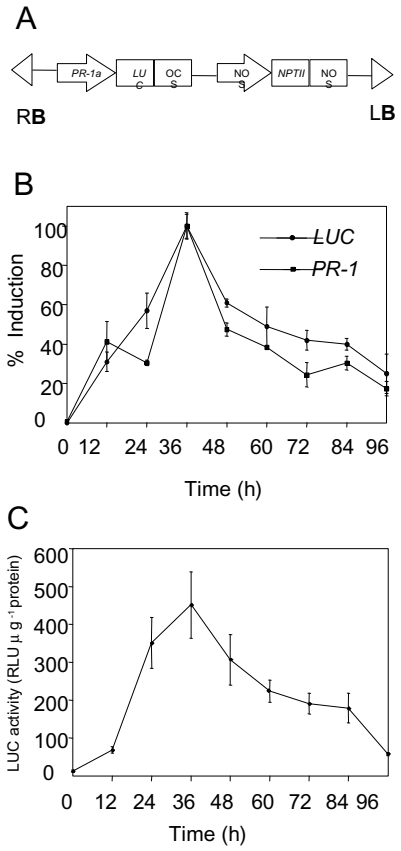

Figure 1. LUC activity following inoculation with *Pseudomonas syringae* pv. *tomato* DC3000

DISCUSSION

We have generated an *Arabidopsis* line containing a chimeric *PR-1::LUC* transgene that robustly reports *PR-1* gene expression during the establishment of *R* gene-mediated disease resistance and SAR. The utility of the novel *PR-1::LUC* transgenic line was exploited in a screen designed to uncover EMS *Arabidopsis* mutants that caused miss-expression of the *PR-1* gene (MURRAY *et al*. 2002). To uncover potentially redundant and/or essential genes integral to the establishment of SAR in *Arabidopsis*, we generated a large collection of activation tagged lines and screened via ultra low light imaging.

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