Genetic and molecular analysis of *Gigantea*, a gene involved in adaptation to climate via regulation of the circadian clock in *Solanaceae*

Análisis genético y molecular de *Gigantea*, un gen implicado en la adaptación al clima a través de la regulación del reloj circadiano en *Solanáceas*

C. Brandoli*[1], J. Weiss[2], M. Egea-Cortines[2]

[1] IBV, Institudo de Biotecnología Vegetal, Universidad Politecnica de Cartagena, Campus Muralla del Mar, Murcia, Spain

[2] ETSIA, Universidad Politécnica de Cartagena, Paseo Alfonso XIII, 48, 30203 Cartagena, Murcia, Spain

Abstract

The metabolism, physiology, and also the behaviour of most living beings, profoundly changes between day and night. These biological oscillations happen due to the circadian rhythm, an endogenous clock that regulates most of biological events. In the plant kingdom, one of the genes involved in this important regulation is *Gigantea*. Although discovered in the past century, some of its functions, at the molecular level, are unclear and still a source of intense scientific research. In this thesis project we will try to analyze phenotypic and biochemical changes induced by gene silencing in *Solanaceae* with the aim to better understand the function of *Gigantea*.

Keywords: *Gigantea*; recombinant plasmid; gene silencing; RNA interference; CRISPR/Cas9 System.

Resumen

El metabolismo, fisiología y comportamiento de los organismos vivos cambia entre el día y la noche. Estas oscilaciones biológicas se deben al reloj circadiano, un sistema endógeno que regula la mayoría de los procesos biológicos. En el reino vegetal, uno de los genes involucrados en esta regulación es *Gigantea*. Aunque fue descubierto en los años 50, sus funciones biológicas y moleculares no se conocen con detalle y son objeto de intenso trabajo debido a su importancia en la adaptación. En este proyecto de tesis pretendemos analizar cambios fenotípicos y bioquímicos relacionados con el silenciamiento de *Gigantea* en solanáceas.

Palabras clave: *Gigantea*; plásmido recombinante; silenciamiento génico; ARN de interferencia; Sistema CRISPR/Cas9

* E-mail: claudio.brandoli@gmail.com

1. INTRODUCTION

In the last decades, the scientific community has used Arabidopsis thaliana as a model system for identifying genes and determining their functions, as it has many advantages for genome analysis, including a short generation time, small size, large number of offspring, and a relatively small nuclear genome [1].

In more recent years, some genes involved in the regulation of circadian rhythm have been identified in *Arabidopsis*. One of these is *Gigantea*, a gene involved in flowering time regulation, light signalling, hypocotyl elongation, control of circadian rhythm, sucrose signalling, starch accumulation, chlorophyll accumulation, transpiration, herbicide tolerance, cold tolerance, drought tolerance, and *miRNA* processing [3]. It's therefore easy to understand why we chose to analyze the molecular pathway of this very important gene.

The following thesis project has different objectives related to the genetic regulation of *Gigantea* in plant crops. Between these, the phenotypic and biochemical analysis of petunia, an important ornamental plant.

Another objective, linked to the first one, is to silence the *Gigantea* gene through the use of two methods, an RNA of interference and a more recent method, called CRISPR/Cas9, in order to compare and analyze the mutant phenotype with its respective wild type form.

Finally, we obtained transposon-tagged alleles of the genes *Gigantea1* and *Gigantea2* as we found that the gene *Gigantea* is duplicated in *Solanaceae.*

2. MATERIALS AND METHODS

2.1 Analysis and identification of a transposable element in GI1 and GI2.

The first goal of this project that we want to achieve, is to get a plant that will bear in homozygous form a transposon, in the genes *Gigantea1* and *Gigantea2*.

We received from the Laboratoire de Reproduction et Développement des Plantes of the Ecole Normale Superieure de Lyon (ENSL) two lines with transposon insertions *PhGi1::3087dTPH1* and *PhGi2::2995dTPH1* [4]. Hybrid plants were obtained by crossing Mitchell and Lion plants. After having catalogued every plant, we extracted and amplified the DNA from one leaf of each sample, then, we selected the plants carrying the transposon in heterozygous form thanks to a DNA analysis by electrophoresis on agarose gel (see Figure 1).

The plants proved to be in heterozygous form, were self-pollinated in order to obtain a segregation of type 1:2:1 in the second generation, with the aim to select again, only the homozygous forms.

2.2 Selective gene silencing through an interference RNA

We started selecting a region of the cDNA coding for *GI1* and *GI2* that would discriminate between both paralogs. We amplified a DNA fragment by PCR through the use of site-specific primers. This fragment was inserted into a gene vector called pDONR 201 and recombined into pHELLSGATE12 to obtain hairpin-like structures (see Figure 2).

Transgenic plants will be produced with the specific silencing of *GI1* and *GI2*.

2.3 CRISPR/Cas9 System

It is a new and innovative method for genome editing, enabling the precise manipulation of specific genomic sequences. This new technology relies on one sequence-specific nuclease called Cas9, used to generate double strand breaks at any desired location in the genome, thanks to a single guide RNA (sgRNAs) which "leads" the nuclease to the target sequence (see Figure 3).

As illustrate in Figure 3, some of the possible applications are (1) simultaneous target mutagenesis at multiple loci, (2) targeted chromosomal deletion, (3)synergistic or tunable deletion of a gene of interest, (4) synergistic or tunable repression of a gene of interest, (5) simultaneous activation of multiples genes and (6) simultaneous repression of multiples genes [2].

3. Acknowledgments

I would like to thank the Institute of Plant Biotechnology (IBV), especially my director and co-director of thesis, Dr. Julia Rosl Weiss and Dr. Marcos Egea Gutiérrez-Cortines for their willingness and their valuable advice. This works is part of projects BFU-2013-45148-R and Seneca 19398/PI/14

4. REFERENCES

[1] Arabidopsis, T., & Initiative, G. (2000). Analysis of the genome sequence of the ¯ owering plant Arabidopsis thaliana, *408*(December).

[2] Lowder, L. G., Zhang, D., Baltes, N. J., Iii, J. W. P., Tang, X., Zheng, X., … L, N. C. L. G. (2015). A CRISPR / Cas9 Toolbox for Multiplexed Plant Genome Editing and Transcriptional Regulation 1 [OPEN], *169*(October), 971–985. http://doi.org/10.1104/pp.15.00636

[3] Mishra, P., & Panigrahi, K. C. (2015). GIGANTEA – an emerging story, *6*(January), 1–15. http://doi.org/10.3389/fpls.2015.00008

[4] Van den Broeck, D., Maes, T., Sauer, M., Zethof, J., De Keukeleire, P., D'hauw, M.,... Gerats, T. (1998). Transposon Display identifies individual transposable elements in high copy number lines. The Plant Journal : For Cell and Molecular Biology, 13(1), 121–9. doi:10.1046/j.1365-313X.1998.00004.x

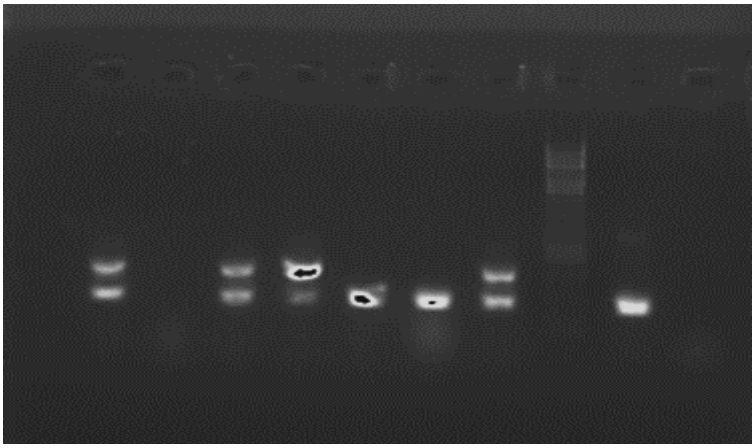

Figure 1. Identification of heterozygous forms in 7 different samples of plants, agarose gel 1%

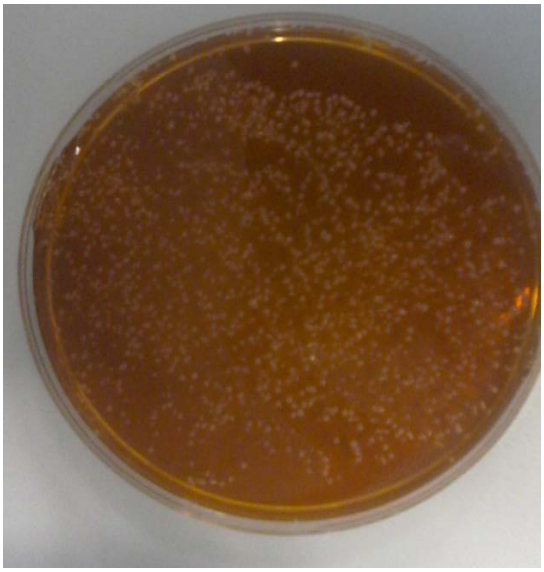

Figure 2. *Agrobacterium tumefaciens*, carrying fragment of Gigantea1 gene in a hairpin-loop.

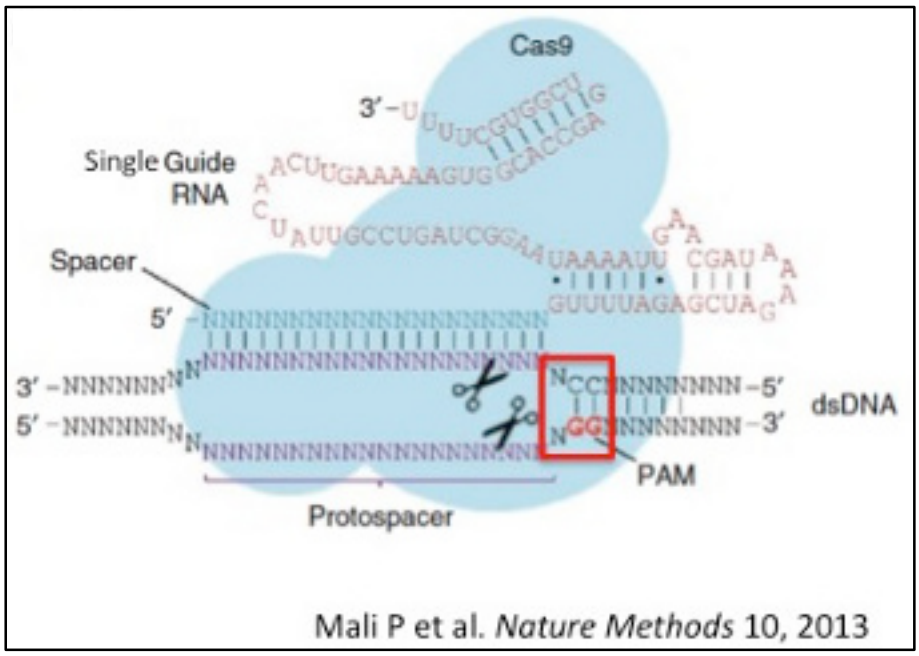

Figure 3. The CRIPR/Cas9 system