Development of loss of function alleles based on CRISPR/CAS9 to study flower and fruit development
Desarrollo de alelos de pérdida de función basados en CRISPR/CAS9 para estudio de desarrollo de flor y fruto

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Abstract

The CRISPR/cas9 system has taken over the methodologies to obtain new alleles as it is based on DNA repair mechanisms activated locally by DNA breakage. The CRISPR/Cas9 complex is driven by a guide RNA conferring specificity of action on a given DNA sequence. The aim of the work is to develop a set of alleles in genes of interest involved in flower and fruit development.

Keywords: Genetic engineering; developmental genetics.

Resumen

El sistema CRISPR/Cas9 se ha convertido en la tecnología más útil para obtener alelos nuevos al estar basada en el proceso local de reparación de ADN activado localmente por la rotura del ADN. El complejo CRISPR/Cas9 es guiado por una molécula guía de ARN que le da especificidad de acción sobre una secuencia de ADN concreta. El objetivo del trabajo es desarrollar una colección de alelos en genes de interés relacionados con el desarrollo de la flor y fruto

Palabras clave: Ingeniería genética; genética del desarrollo.

1. INTRODUCTION

Since the beginning of biological research, scientists aim to develop new gene editing technologies for prospering genome manipulations. The adoption of programmable nucleases in the advancement of genome editing technologies have significantly improved the ability to successful changes in eukaryotic genome.

To date, there are different types of gene editing strategies developed such as, meganucleases, Transcription Activator-Like Effector Nucleases (TALENs), Zinc Finger Nucleases (ZFNs) and the CRISPR-Cas9 system [1], [2]. Unfortunately, TALENs and ZFNs systems are mainly based on protein-DNA interactions which requires cloning of new protein for each target site [3]. Besides, by simply altering the guide RNA, Cas9 system can aim towards a new target location. Therefore, this feature makes CRISPR/Cas9 system a crucial element of gene editing strategies.

It has been shown that the CRISPR/Cas9 technology has been successfully used in Petunia and tomato [4][5] [6]. The aim of the PhD proposal is to develop a set of CRISPR/Cas9 based alleles in genes of interest for flower and fruit development. Antirrhinum, Petunia, tomato and Arabidopsis plants will be studied.

2. MATERIALS AND METHODS

Antirrhinum, petunia, tomato and Arabidopsis transformation protocols has been described below.

2.1 Gene Database development

The flowering and fruit development related genes of Antirrhinum, Petunia, Tomato and Arabidopsis plants will be selected and obtained from previous studies of our laboratories and online databases.

2.2 Plant Material

The *Petunia hybrida*, *Antirrhinum majus, tomato* and *Arabidopsis thaliana* will be grown in growth chamber conditions and the full development will be held in greenhouse followed by transformation of CRISPR/Cas9 technique. Seeds of *Petunia hybrida* line Mitchell and *Antirrhinum majus* will be surface-sterilized. The seeds will be sowed on Murashige and Skoog medium (Duchefa, Haarlem, Netherlands) and will be solidified with 4 g/L of Phytagel (Sigma-Aldrich, Madrid, Spain). The samples will be placed on growth chambers under a photoperiod of 12/12 h of light/dark and 25 °C/18 °C temperature.

2.3 Vector Construction

The vector construction will be established by conventional molecular cloning procedures. gRNAs and PCR primers will be created for selected gene families.

2.4 Transformation (Agrobacterium)

Transformation will be conducted by using *Agrobacterium tumofaciens* through suitable vector including the gene of interest for each specific context.

3. RESULTS AND DISCUSSION

The correlation between the genes of interests and the developmental stages, especially circadian clock related pathways, of selected plants are aimed to be observed.

4. CONCLUSIONS

The simplicity and sustainability of CRISPR/Cas9 system makes it a valuable genome editing tool amongst other genome editing methods. The gene editing techniques having the ability to accelerate plant breeding by enabling the precise gene modifications. CRISPR/Cas9 technology is specifically important amongst all other gene editing technologies due to its ability to modify multiple traits simultaneously [7]. Moreover, the technique does not require any protein engineering processes.

In this PhD thesis, we aim to obtain CRISPR/Cas9 based alleles of Antirrhinum, Petunia hybrida, Tomato and Arabidopsis plants targeting the flower and fruit development genes.

5. ACKNOWLEDGMENTS

The experiments will be conducted in Institute of Plant Biotechnology (IBV), Cartagena within Polytechnic University of Cartagena.

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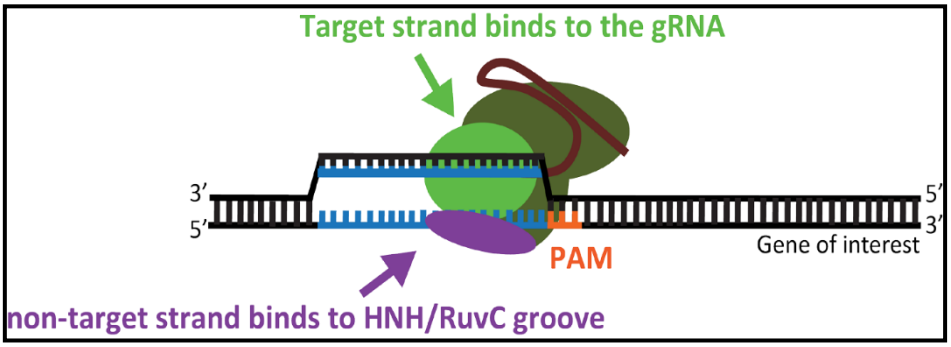

Figure 1 The representative image of RNA guided DNA cleavage by CRISPR/Cas9 system